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THE JAPANESE JOURNAL OF CANCER RESEARCH

Founded by K. YAMAGIWA and Continued by M. NAGAYO

Vol. 45, No. 4 December 1954

Published By
THE JAPANESE CANCER ASSOCIATION AND
THE JAPANESE FOUNDATION FOR CANCER RESEARCH
Nishi-Sugamo, Toshima-ku, Tokyo, Japan

Subscription Price for Foreign Countries $3.00 per Volume Post Free

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[GANN, Vol. 45; December, 1954]

ON HISTOGENESIS OF CERVICAL CANCERS OF THE UTERUS. A HISTOLOGICAL STUDY ON *IN SITU* AND EARLY CARCINOMAS. (With Plates VI—IX)

KUNIO OOTA and MAKOTO TANAKA
(Cancer Institute and Tokyo Medical and Dental College)

On the origin of cancers of the cervix of the uterus two major assumptions have been prevailing: many believe that they usually originate from the squamous epithelium of the portio, while a few consider them as derivatives of cylindric epithelium of the cervix. Both groups of authors unanimously admit that the squamo-columnar junctions are predilected as the primary sites of cancerization.

R. Meyer (1930) was of the opinion that cervical carcinomas arise either from the squamous epithelium or from the columnar epithelium, assuming that similar solid alveolar structures of cancers resulted irrespective of their exact origination. There, it seems, have been no dependable data in the literature as to how frequently solid cancers develop from one of those two kinds of epithelium.

Early cancers are undoubtedly most suitable for the purpose of determining frequency of their origination from either one of the epitheliums. Foote and Stewart (1948), in their study of *in situ* carcinomas of the cervix, stated that among 17 of their thoroughly sectioned cervices, 10 such lesions are limited to the portio vaginalis and 3 to the endocervix, the rest occupied both the portio and endocervix. Generally speaking their materials showed concentration of the lesions in a critical area about the external os.

Howard et al. (1951), on the other hand, suggest columnar origin of the majority of cervical carcinomas and pointed out the similarity between histogenesis of squamous metaplasia of the endocervical columnar epithelium and cancerization. Squamous metaplasia of the cervical epithelium has been found between 70 and 90 % of all adult cervices examined by many (especially by Carmichael and Jeaffreson (1939)). R. Meyer believed that it represents a remnant of embryonic squamous epithelium or direct extension of portio lining epithelium in the course of healing of an erosion. Frequent occurrence of such squamous epithelium in the endocervix obscures the argument on epithelium in cancerization.

Material and method: 56 early carcinomas were found among many thousands of cervical lesions examined histologically during the period from 1949 to 1953. Four showed no remnant of cancer after positive biopsies. In all 203 uteri

were resected, 147 for advanced cancers, during the same time period. The entire portio and distal cervices were cut systematically in a way similar to that described by Foote and Stewart, although relatively small dimension of the portiones of Japanese women allowed only to take 12 or 14 blocks from each of them. Two additional transverse sections were taken from the middle and upper two-third levels of each cervix to know the upper extension of the lesions. Each block was embedded in paraffine and cut stepwise or serially.

H and E, Mayer's mucicarmine, Best's carmine, PAS stain of McManus, metachromatic staining with toluidin blue, Mallory, elastica-v. Gieson, and Pap's silver impregnation method were used.

101 cases of advanced cancer, not seriously influenced by previous treatments, and at random examples of 100 from over 4000 biopsies of good size, were also examined in parallel.

Results

1. The early cancers are listed in Table 1, classified according to the extent of the lesions. The term *carcinoma in situ* was used to designate well delineated atypical hyperplasia of the epithelium through full thickness with dedifferen-

Table 1. Early carcinomas.

In situ carcinomas	without glandular involvement		0 ※	26	52
	with glandular involvement	Stage I	15		
		Stage II	6		
		Stage III	5		
Microcarcinomas			11	26	
Small carcinomas			15		
Cancer *in situ* only in biopsies			4 ※		4
Total					56

※ In one case included in the last category, intraepithelial cancer without glandular involvement was seen.

tiation and disorganization, hyperchromatism, variegated size and mitotic activity of nuclei, and increased nucleo-cytoplasmic ratio, but without overstriding any part of the basement membranes, either of the surface or of the glands. They are classified in 4 series. None was found without glandular invaslon to fall into the first category, except one in which operative material after positive biopsy showed no remnant of cancer. In over half of our materials several glands are entirely filled by cancerous epithelium and their basement membranes are distended (stage 3). Glandular invasion of lesser degrees (stages 1 & 2) was found in the rest of materials (Fig. 1).

Lesions with general features of carcinoma *in situ* above, accompanied by a

single focus of minute invasion into the stromal tissue, were classified in the group of microcarcinoma (Held) (Fig. 2).

More advanced condition with a larger focus of multiple invasive foci, but limited around only a few glands and not forming ulcers, was put into the category of small carcinoma (Fig. 3). All lesions invariably contained far more intraepithelial than stromal infiltration. None had ulcer except at the sites of previous biopsies.

2. Localization and distribution of the lesions:

Distortion of gross structures, mainly due to parturitional injuries, and alteration of microscopic appearance in the healing stages of erosion (R. Meyer), very frequently cause difficulties in determining the physiological junctional zones. Roughly they are characterized with the most distal "physiological" endocerical gland subjacent to them. Extension of definite squamous epithelium upward from this zone is very irregular and intervened by columnar epithelium generously. Secondary formation of glandular structures may extends even to the periphery of a portio, but it can be differentiated with relative ease from the physiological ones. It is of note that our squamous region is no narrower than the "portio" of Foote and Stewart.

In the majority of the early cancers both columnar and squamous regions are affected, i. e., in 44 cases among 52 or 84.5 % (Table 2). 101 advanced cancers examined in the same period involve these both areas 86 times (85.1 %).

Tendency of *in situ* cancers to involve more of the columnar than of the squamous epithelial regions is clearly shown in the Table 2. Exclusive affection of the columnar region was in 6 cases as compared with one case of exclusive involvement of the squamous epithelial region. This relation is obscured in the advanced cancer group, in which ratio between lesions limited in the columnar and squamous region is 10 : 8. If the cases with affection of the adjoining areas in Table 2 were added to the above figures, ratio between the squamous and columnar predilections makes in early cancers 10 : 29, where as in advanced cancers it is 37 : 40.

3. Spread of *in situ* lesions:

In several *in situ* lesions the carcinomatous epithelium appeared to have extended, not only replacing and displacing the normal pre-existent epithelium, but also covering *de novo* the previously eroded surfaces.

In case replacement of smooth surface epithelium has occurred, resulted carcinomatous covering show likewise a smooth glistening surface, grossly normal at casual observation (Fig. 4). Fresh lining of eroded areas with cancerous epithelium usually offers an impression of pseudoerosion. This seems to be important, partly because pseudoerosion and carcinoma *in situ* often appear in the corresponding region, and partly because this fact strongly suggests the

possibility of cancerization in the course of preudoerosive reparation of chronically inflammated areas.

Table 2. Localization of early cancers:

		Ca. *in situ*	Micro-ca.	Small ca.	Ca. only in biopsy	Total
Exclusive involvement of squamous region		1	0	0	0	1
Both squamous and columnar regions involved.	More squamous region involved	1	4	4	0	9
	Sq. & column. regions equally involved	6	2	5	0	13
	More columnar region involved	12	4	5	0	21
Exclusive involvement of columnar region		6	6	1	4	12
Total		26	11	15	4	56

In situ cancers spreading over eroded surfaces come in contact in the periphery with normal squamous epithelium to show a sharp and abrupt difference in thickness on both sides of the juncture. Cancerous epithelium is usually far thinner than benign one (Fig. 5). Pre-existent squamous epithelium replaced by an *in situ* cancer does not differ in thickness (Fig. 6).

Intervening columnar epithelium was often noticed between squamous epithelium and spreading front of *in situ* carcinoma arising from the glandular endocervical region (Fig. 7).

4. Histology of early cancers and their localization:

Tables 3 and 4 are shown to list possible correlation between histological types of early cancers and their territorial distribution. Cellular patterns are described on purely morphological basis (Oota, 1949). Unlimited use of such designation as squamous or epidermoid carcinoma is avoided, mainly because such terms often erroneously suggest origin of the cancer cells.

As has been suggested by R. Meyer mucicarmine stain was of great help to detect trace of mucus-secreting ability retained in cancer cells, which are to be designated as columnar type in this paper (Figs. 8 and 9). To avoid errors due to contamination of cancer cells with mucin, secreted by adjacent normal glandular epithelium, special caution was paid.

Negative mucin finding does not exclude columnar cell character of the tumor cells. A proliferating part of tumor tissue, very often fails to show such specific activity, even if the cancer as a whole may be well differentiated. In one of the small cancers and three of advanced cancers, endolymphatic cancer cells only were mucin-positive components in the whole tumor tissues. In this meaning, mucin-negative and keratin-negative *in situ* carcinomas are not necessarily

Table 3. Histological differentiation of *in situ* carcinomas in relation to localization.

		No. of case	Cellular pattern	Specific differentiation		Type of cancerous epithelium.		
				Keratinization	Mucin secretion	Sq. epith.	Undecided	Columnar epith.
Exclusive involvement of squamous region		G 240	p. -sp.	+	—	sq.		
Both squamous & columnar regions involved	More squamous region involved	G 67	p. -sp.	+	—	sq.		
	Both squamous & columnar regions equally involved	G 197	f. -p.	—	—		un.	
		〃 211	f. -p.	±	—	sq.		
		〃 467	p.	+	—	sq.		
		〃 477	p.	—	—		un.	
		〃 483	p. -sp.	±	—	sq.		
		〃 490	p. -sp.	+	—	sq.		
	More columnar region involved	G 175	f. -p.	—	—		un.	
		〃 217	f. -p.	—	—		un.	
		〃 303	f. -p.	—	—			co.
		〃 308	f. -p.	—	+			co.
		〃 336	p. -sp.	++	+	sq.		
		〃 360	f. -p.	—	—			co.
		〃 450	f. -p.	+	±	sq.		
		〃 457	f. -p.	—	—		un.	
		〃 470	f. -p.	—	—			co.
		〃 473	p.	—	±			co.
		〃 488	f. -p.	—	—		un.	
		〃 496	p. -sp.	±	—	sq.		
Exclusive involvement of columnar region		G 39	f.	—	±			co.
		〃 143	p.	—	—		un.	
		〃 156	f. -p.	—	—		un.	
		〃 300	f. -p.	—	±			co.
		〃 390	p. -bulb.	±	—	sq.		
		〃 394	f. -p.	—	—		un.	
		〃 469	p.	—	—		un.	

Table 4. Histological differentiation of microcarcinomas and small carcinomas in relation to their localization.

		No. of case	Cellular pattern	Specific differentiation		Type of cancerous epithelium		
				Keratinization	Mucin secretion	sq. epith.	undecided	Columnar epith
Exclusive involvement of squamous region		—						
Both squamous & columnar regions involved	More squamous region involved than columnar	G 40	f. -p.	∓	−	sq.		
		〃 59	f. -p.	−	−		un.	
		〃 64	f. -p.	−	+			co.
		〃 90	f. -p.	−	±			co.
		〃 118	f. -p.	+	−	sq.		
		〃 231	p. -vac.	−	±			co.
		〃 245	sp.	+	−	sq.		
		〃 295	f. p.	−	−		un.	
	Both squamous & columnar regions equally involved	G 57	f. -p.	−	−		un.	
		〃 69	f. -p.	−	−		un.	
		〃 404	p.	±	−	sq.		
		〃 455	p. -vac.	−	+			co.
		〃 460	p. -sp.	+	−	sq.		
		〃 485	sp.	±	−	sq.		
		〃 347	p. -sp.	+	+	sq.		
	More columnar region involved than squamous	G 2	f. -p.	−	−		un.	
		〃 147	f. -p.	−	−		un.	
		〃 256	p.	−	+			co.
		〃 299	f. -p.	−	−		un.	
		〃 325	f. -p.	−	±			co.
		〃 407	p. -sp.	+	−	sq.		
		〃 414	p.	−	+			co.
		〃 464	p.	±	∓			co.
		〃 495	p. -sp.	±	−	sq.		
Exclusive involvement of columnar region		G 124	p.	−	−		un.	
		〃 196	f. -p.	−	−		un.	
		〃 213		−	−		un.	
		〃 249	p.	−	+			co.
		〃 426	p. -vac.	−	−		un.	

highly anaplastic, only because of lacking specialization.

Keratinization and spinous structures are arbitarily used as criteria to designate cancers as of squamous cell type. That it does not mean squamous origin, will be discussed later. Designation of the cancerous epithelium, either as squamous or as columnar, was possible in 60 to 70 % of the present series with fairly good objectivity. Equivocal lesions were left unclassified.

Unexpected clear cut correlation was seen in this tabulation between predominance of one histological type or other, and localization of the lesions.

In the series of *in situ* carcinomas, mucin-positive tumor tissues are seen exclusively in the groups, in which pure or predominent involvement of the columnar region was observed (Table 3). More diffuse territorial distribution of such type of cancers was noted in the microcarcinoma and small carcinoma-series (Table 4). Advanced cancers, which included 39 such tumors, showed the same tendency as in early cancers, but again with distinct preference of the columnar region by mucin-positive tumors (in squamous region 11, in columnar region 18).

Squamous type cancers with positive keratinization and spinous structures were more diffusely distributed in the early series (Tables 3 & 4). They were seen in almost any area, but more frequent appearance in the squamous and junctional regions is suggested. A squamous carcinoma with exclusive involvement of the columnar area was observed in one cese of microcarcinoma. Among

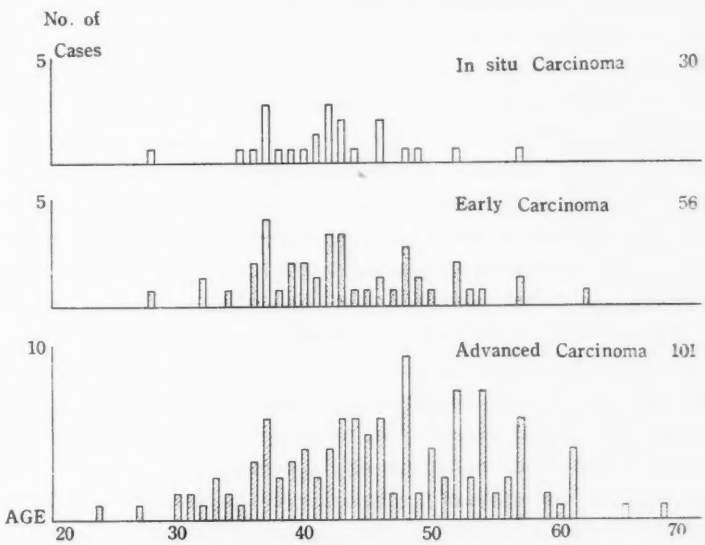


Chart 1. Age Incidence of Uterine Cancer

31 advanced cancers of squamous cell type, almost equal incidence of the lesion in squamous and columnar areas was noted, quite parallel with the above finding on *in situ* cancers: seven advanced cancers of squamous cell type involved exclusively the endocervix. It is of note that in advanced series some of mucin-positive cancers (5 among 28) showed simultaneously keratinization.

Age incidence:

Age incidence of the *in situ* carcinoma, at the total of early cancers, including *in situ*, micro- and small carcinomas, and of the advanced cancers of the cervix resected in the same period, is tabulated (chart 1). Tendency of earlier occurrence of *in situ* carcinomas compared with incidence of advanced cancers is clearly demonstrated, which is in coincidence with data of earlier workers (Pund et al., Galvin et al. ect.). It is also shown that carcinoma *in situ* can occur at any age, the youngest being at 28 and the oldest at 57 of age in the present series.

Discussion

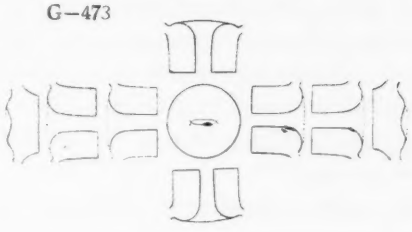
There seems to have been a definite inclination to postulate that all squamous or epidermoid carcinomas to arise from squamous epithelium. It is especially true with the cervical carcinoma in question. Hinselmann was definite to incriminate leucoplakia for the origin of all cervical cancers. Ewing and Stone also thought they originate from precancerous hyperplasia of squamous epithelium. But, Schiller (1927) pointed out that *in situ* carcinoma occurs in unchanged squamous epithelium, without antecedent hyperplasia.

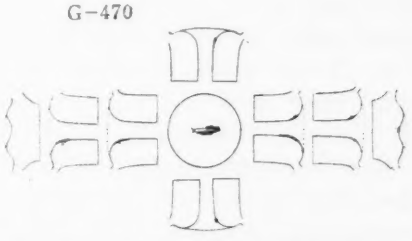
Cervical cancers involve in the majority the squamo-columnar junction. Exclusive involvement of the squamous area of the portio vaginalis is rather rare. The latter fact apparently contradicts with the above postulation so far as the distribution is concerned.

To relieve this difficulty, v. Franqué assumed that many cervical cancers arise from the islets of squamous epithelium in the endocervix, which R. Meyer attributed to remnants of embryonic squamous epithelium. Reduplication of mature squamous epithelium by such islets with keratinized surface layers is not uncommon (Carmichael and Jeaffreson, and others). It has been believed since R. Meyer that they represent a healing stage of erosion, and that they are actually extension of the portio epithelium replacing the columnar area. But, as Fluhmann (1953), and Howard et al. (1951) have excellently demonstrated, there are several clues to regard them as products of autochthonous metaplasia occurring in the columnar epithelium of the cervix.

Because of severe distorsion of physiologic gross patterns as well as alteration of epithelial features, which are rather common in the squamo-columnar junctions of the cervices, it is hard to be exact in determining distribution and

extent of the lesions in the classified areas of the cervix. Our criteria for the postulation of juncture in distorted cervix is already mentioned. A three dimensional reconstruction was tried in this study for this particular purpose, as is exemplified in Charts 2 and 3.





Chaits 2 and 3. Examples of three-dimensional reconstruction of the cervical lesions in early carcinomas. Fig. 11 (above) shows a carcinoma *in situ* of columnar epithalial origin and with very limited extension. Fig. 12 (below) represents a more advanced phase of carcinoma *in situ*, also of columnar epithelial origin, involving both the anterior and posterior lips. The lesion appears to have spread by contiguity from a single origin.

According to Foote and Stewart (1948), exclusive involvement of the portio squamous area was seen in 60 % of cases among their series of 17. Our series, in contrast, revealed predominent endocervical localization of *in situ* carcinomas and other early cancers. Gross features of the majority of advanced cancers of the cervix, with more destruction of the endocervix than the portio, might more easily be understood, if their supposed origins were situated somewhere in the endocervix rather than at the portio vaginalis.

Some of the early cancers in the present study, which appear in columnar area are definitely squamous in character, and possibly so also in origin, but others differ from the former group considerably in their histologic details. It is of note that the columnar cell type tumor is rather rarely seen in the squamous region of the cervix.

R. Meyer appears to have avoided the term "epidermoid" and designated the maiority of cervical cancers as solid carcinomas. He also stressed amphoteric origin of these cancers both from squamous and glandular epitheliums, although he put more weight to the former. Possibility of differentiation of their origin with mucicarmine staining was also suggested by him.

Gradual transition of a definite endocervical adenocarcinoma into a solid

carcinoma, closely simulating squamous nature, is not uncommon. The solid "epidermoid" alveolar structure, when independently examined, will almost exactly recall a squamous carcinoma. Some such examples are demonstrated in the following few figures (Figs. 10, 11 and 12).

In the salivary gland a special type of tumor has been called attention by Stewart, Foote and Becker (1945), They coined a term "mucoepidermoid tumor" for this group. Coexistence of epidermoid features with muciferous differentiation of tumor elements offers close similarity with some type of adenocarcinoma of the cervix merging into epidermoid pattern, although Stewart is not inclined to include such type of cancers in their category.

Ringertz (1937) has shown how a solid carcinoma, closely resembling squamous type, arises from columnar epithelium of the nasal and paranasal mucosa. We interpreted this process as metaplastic metamorphosis occurring in a malignant tumor.

Black and Ackerman (1952), in reporting and stressing the importance of *in situ* carcinoma in the histogenesis of bronchogenic carcinoma, stated that squamous metaplasia antecedes cancerization. If this be true also in all "epidermoid" carcinomas of the endocervix, they should show more definite squamous characteristics with complete absence of mucus-secreting activity.

In this concern, a close similarity between so-called epidermization of columnar epithelium occurring in non-tumorous endocervix on one hand, and metaplastic metamorphosis of an originally mucinogenic carcinomas into an epidermoid type on the other hand, deserves special consideration. There are at least two types of epidermization of non-cancerous columnar epithelium. One is a type leading to a complete transformation of columnar epithelium into squamous one (Fig. 13). This occurs, contrary to the opinion of R. Meyer, in adult women and autochthonously. The other type represents actually a stratification of columnar epithelium with irregularly distributed micinophorous cells in various layers (Fig. 14). The latter duplicates to a great extent what we call metaplastic metamorphosis in cancer of columnar cell origin, as Howard et al. have already pointed out.

Both in the epidermization of normal columnar epithelium and metaplastic metamorphosis of cancers, true squamous differentiation can occur. Thus the squamous character is appearently short in qualifiying an epithelium as epithelium as originally squamous, whereas mucin-producing character is enough to designate them as columnar or glandular origin. In spite of common origin of both endocervical and vaginal epithelium from Muellerian duct lining, advers transformation, "adenomatization" of a squamous epithelium, appears never to have been observed.

Here we have no intention to go into the natural history of *in situ* carcinoma

of the cervix. Younge et al. found in their retrospective studies, that their intraepithelial hyperplasia, or basal cell hyperactivity of the other authors, precedes *in situ* carcinoma. As Schiller already stated, there seems to be no hyrertrophic or hyperkeratotic changes in the epithelium which is doomed to develop carcinoma *in situ* later, It is preferred to avoid the ambiguous term "precancer", because some such precancers of earlier workers seem to represent already established maligancies *in situ* in our present interpretation.

We have arbitarily divided our materials of early cancers in three categories, but we do not know any chronological squence of carcinoma *in situ*, as to whether or when it will begin to grow with infiltrative tendency. What we know at present is that it shows clear cut lateral invasion of adjacent and subjacent structures.

Wespi has recently postulated that cervical cancers in women younger than 50 might have longer latency, substantiated in the form of carcinoma *in situ*, whereas women of older blackets develop cancers which immediately commence stromal invasion. He stated that he never observed carcinoma *in situ* in the latter age group of women. Age incidence of our present material does not agree with him. It includes two cases of *in situ* carcinomas above 50, and far advanced cancers in young women of 24 and 27.

Overall incidence of cervical cancers among Japanese female population is said to be higher as compared with date from the Western countries. If a carcinoma *in situ* actually represents preinvasive stage of an invading cancer, higher incidence of carcinoma *in situ* should be expected in the same population than in tne others. Unfortunately enough, comparable data are rather labile. Figures reported from a single institution sometimes differ from each other considerably. Only conclusion in this concern is that carcinoma *in situ* is not infrequent also among Japaneses women.

Summary and Conclusion

1. A systemic histological study of 56 early carcinomas of the uterine cervix, including 26 *in situ* carcinomas, is presented.

2. Predominent localization of the early malignant lesions in the columnar epithelial region of the cervix was observed.

3. Many early cancers showed faint mucicarminophilic droplets. (28.6 %) In the advanced cancer series mucin-positive solid carcinomas comprised an unexpectedly high percentage (28 : 101=27.7 %). On the other hand, clear cut transition between definite adenocarcinoma, and solid "epidermoid" carcinoma was observed. If the latter group is included, mucin-positive "epidermoid" carcinomas comprise 34.7 % of all cervical cancers.

4. The above observation led to a conclusion that almost one-third of the

cervical carcinomas are definitely of columnar epithelial origin.

5. The process can be understood as metaplastic metamorphosis, either in the course of cancerization or after cancerization of the columnar epithelium of the endocervix.

Acknowledgement: The authors are indebted to the members of the Gynecological Clinic of the Cancer Institute, Tokyo, for the materials, and to all of our Pathology Laboratory for technical assistance.

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Addendum: Gusberg et al., on the basis of histological examination of 66 resected uteri, state that 66 % of their material had involvement of the cervical canal alone, and in none of their cases exclusive involvement of the portio was seen. Their data appear to be in agreement with our opinion.*

* Gusberg, S. B., S. A. Fish and Yin-Ying Wang: Obst. Gyn. 2 (1953): 557, cited from Intern. Abstr. Surg. 99 (1954): 165

要　旨

子宮頸部癌の組織発生について，上皮内癌及び早期浸潤癌の組織学的研究

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子宮頸部癌の組織発生については，従来扁平，円柱上皮境界が重視され，ことに扁平上皮に重点が置かれて来た。また円柱上皮起原を重視する学者中にも，まずその扁平上皮化生があって，そこに発癌するとの解釈が多数を制する傾きを示してきている。これに反して著者等は例の早期癌手術子宮中，26 例の上皮内癌をも含め，その原発部位が，主として円柱上皮領域内にあること，円柱上皮癌の癌化後の化生的機転が，子宮癌に最も頻度の高いいわゆる「類表皮癌」の組織像に導くものであること，の二つの具体的証拠を提出して，子宮頸部癌の発生母地中，円柱上皮起原を強調する。進行した 101 例の癌についても同様の結論が適用できることを認めた。

Fig. 1. *In situ* carcinoma with limited involvement of glands.

Fig. 2. Microcarcinoma: an early carcinoma with extensive *in situ* lesion and a single focus of beginning invasion in the underlying stroma.

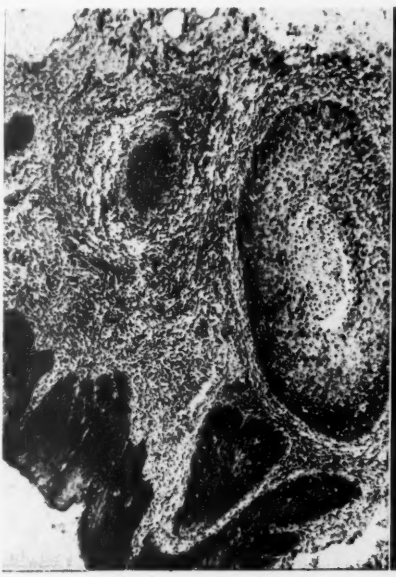

Fig. 3. An example of "small carcinoma" in this series, showing extensive intraepithelial lesion with involvement of glands and limited but more advanced invasiveness than in a microcarcinoma, as seen in Fig. 2.

Fig. 4. Carcinoma *in situ* in a woman aged 49. On gross examination the portio appears almost normal, The squamous epithelium has been replaced by cancerous epithelium of exactly the same thickness, as is shown in Fig. 6.

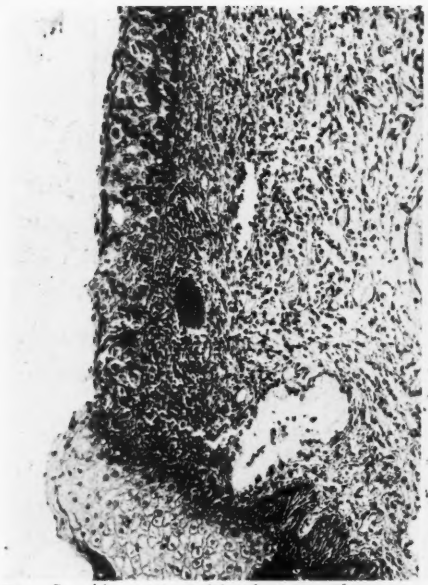

Fig. 5. Abrupt transition between the normal squamous and malignant epithelium. Note the step formation. In this case an eroded surface has been covered by extension of carcinoma *in situ*.

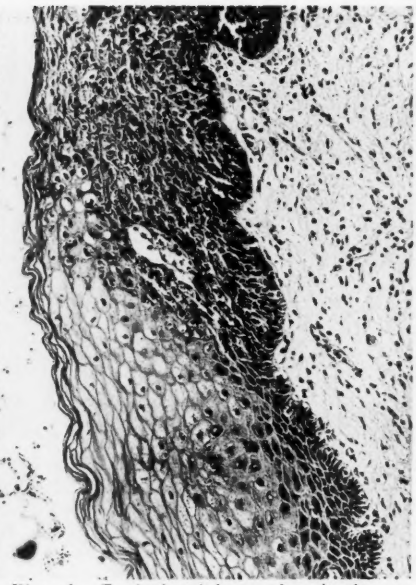

Fig. 6. Typical peiphery of an *in situ* carcinoma. There is no difference in the thickness of epithelial layer between the normal and malignant areas.

Fig. 7. Islet like occurrence of an *in situ* lesion in a polypoid endocervical mucosa. The malignant epithelium appears definitely in the columnar epithelial area without any relation with squamous epithelium, normal or metaplastic.

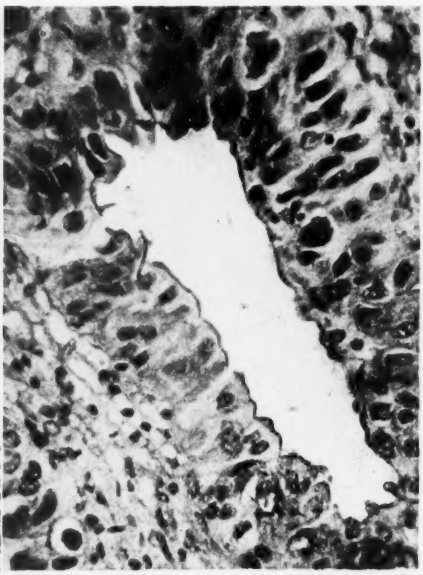

Fig. 8. Mucicarminophilic droplets in the cancerous epithelium. The entire lesion was classified as a small carcinoma in this series. Near the orifice of the cervix it has grown in a typically "epidermoid" pattern, while high up in the canal several alyers of such columnar cells surround a lumen, and might well be called an adenocarcinoma.

Fig. 9. An example of an advanced carcinoma of the cervix with almost exclusive solid alveolar pattern. Irregular distribution of cancer cells with definte mucicarminophilic droplets is clearly demonstrated.

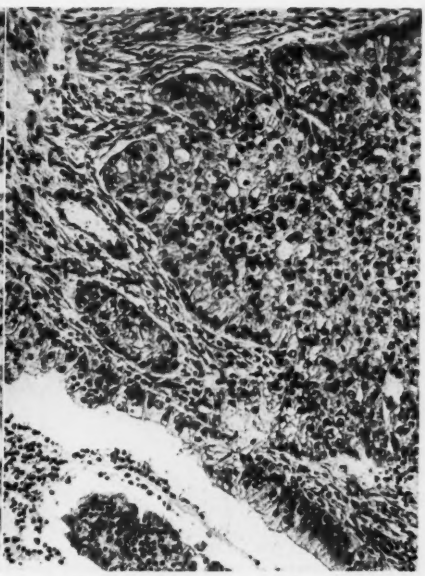

Fig. 10. Adenocarcinoma of the endocervix in an early stage of transformation into epidermoid type.

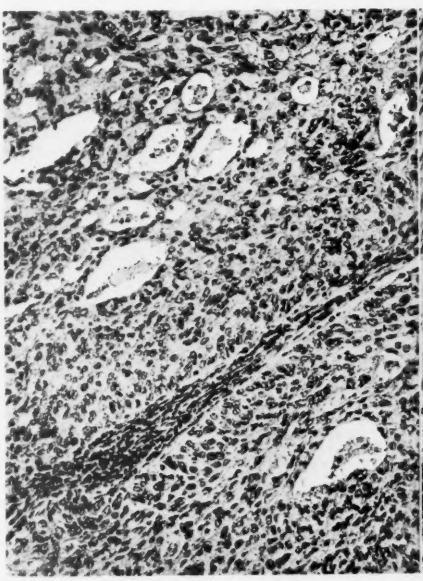

Fig. 11. Adenocarcinoma of the endocervix with a large solid nest closely resembling an "epidermoid carcinoma".

Fig. 12. Adenocarcinoma of the endocervix with prominent mucin-producing character in the right lower hand of the field. More marked epidermoid features are demonstrated in the other parts.

Fig. 13. Autochthonous trasformation of columnar epithelium into squamous type high up in the endocervix near the isthmus in a 41 year-old woman without cancer.

Fig. 14. Another type of epidermization of non-cancerous epithelium of the endocervix, representing stratification of columnar cells with irregularly distributed mucinophorous elements.

[GANN, Vol. 45, December, 1954]

COLLISION OF MULTICENTRIC CANCEROUS FOCI AGAINST EACH OTHER IN EXPERIMENTAL RAT LIVER CANCER

(With Plates X-XIV)

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Not a few tumor pathologists are of the opinion that multiplicity of tumorigenesis is common in man. Multiple tumors of one organ have been frequently reported. On the other hand experimental oncologists have already presented data to show that cancer arises in a group or groups of cells when carcinogenic stimuli are applied to a certain area.

Willis has introduced a concept of field cancerization, especially in view of skin cancers in man. Most authors who favor Willis' opinion, believe that in tumorigenesis disseminated foci of cancerization occur in a limited field, and that cancer grows at first by way of multiplication of cancer cells at many disseminated centers crowded in a restricted area, and further, that a single nodule of macroscopic cancer is established by way of coalescence of multicentric microcancers. Concept of assimilatory growth of a cancer, once rejected by most modern pathologists, appears to have been given a new interpretation and revival. Pleomorphism of tumor histology is also given some kind of explanation in this way.

One of the present authors (K.O.) has, in a previous report, expressed his opinion concerning the generalization of the above concept. Detailed follow up of fate of each microcancer and also study of interrelation of the microcancers in a restricted field of cancerization have been apparently lacking so far. If it is true that several microcancers coalesce into an unit cancer which grows subsequently as an entity of malignant tumor, it necessarily follows that either those several original microcancers can be mixed up homogenously despite their heterologous origins, or they are originally homologous from the instant of cancerization despite their multicentricity. Another possibility is that the process of assimilation includes modification of genotypes of other microcancers by a main tumor.

It is very unlikely, on the basis of mutagenic concept of malignancy, that multicentric foci of cancerization undergo a single directed mutation to form homologous cancers in different foci. If a mixture of heterologous tumor cells is the constituent of a single tumor nodule, it may be recognized in histology,

either of the original tumor nodule or of its successive transplants.

To us it appears controversial to admit the mutagenic theory of carcinogenesis on one hand, and the concept of the assimilation of different microcancers to a single homologous entity on the other hand.

Our fundamental working hypothesis is that, if a cancer cell were a mutant of a normal tissue component, it originates from a single cell, and that different tribes of cells might not coalesce into a single nodule of tumor usually. Exceptional cases may represent a mixed tumor of combination or composition type of R. Meyer.

Tentative examples of early collision of two tumors in the stomachs were produced in the earlier reports, but we have found it extremely difficult to exemplify such phenomena in human materials. Observation on animal organs, which have potency to produce more than one type of malignant tumors in experimental carcinogenesis, seems to be more suitable to simplify interpretation of that debatable process.

Material and Method

Stock ablino rats (105) were devided in three groups: groups R, B & C, were fed rice-diet with 0.06% DAB, butter-diet with 0.06% DAB, and crisco-diet with 0.06% DAB, respectively. The group R consisted of 70 rats, B 10, and C 25. The latter groups of experiment were attempted in order to get more clear cut epithelial hyperplasia and tumors, unaccompanied by mesenchymal reactions, as was stated by Opie (1944). Extreme high-fat diet, margarin or crisco constituting 85 caloric percent, was given to these groups. The results will be discussed elsewhere.

Among 34 rats of group R, surviving at the 150th day of experiment, 27 had at least a single cancer of the liver. Groups B & C with high fat diet were examined at the 300th day of experiment. 2 among 2 of group B, 14 among 15 of group C rats surviving at that time developed at least a tumor nodule, respectively.

Step sections and occasionally serial sections, were cut from above 44 tumor containing livers, H. & E., Mallory, van Gieson, McManus (PAS), and sliver impregnation were applied for histological examination.

Result

(1) Macroscopical multiplicity of tumor nodules were found in high percentage, as seen in the following table (Table 1). Majority of the multinodularity has been caused by metastasis. In advanced cases portal vein thrombosis was frequently encountered.

Many cancerous nodules in a single lobe, or even in several lobes, had similar

structures, at least roughly acceptable as being in the range of widely variable histologic patterns of a single type of carcinoma.

(2) Histologic classification of experimental hepatic malignancies is variegeted according to the authors, apparently because of different interpretation of origin of tubular structures very oftenly encountered in tumor nodules. Maruya (1939) denied to have seen a cancer of bile duct epithelial origin. Yoshida and Sasaki, working with o-amino-azo-toluene, got four pure cholangiocarcinomas (Gallen-

Table 1. Macroscopic numbers of tumor nodules in each hepatic lobe.

Rat No.	Hepatic lobes						Rat No.	Hepatic lobes					
	I	II	III	IV	V	VI		I	II	III	IV	V	VI
R 14	1	1	2	1	1	0	R 45	0	0	3	0	1	0
17	0	2	3	0	1	0	46	1	1	1	1	2	1
20	0	1	0	0	0	0	47	1	1	3	0	2	0
22	0	0	1	0	1	0	48	0	1	1	0	2	0
23	1	0	1	1	1	0	49	1	1	0	1	1	1
24	2	3	4	0	2	1	50	0	2	1	1	1	1
25	1	0	2	2	1	0	51	0	1	1	0	4	0
26	1	0	1	1	1	1	B 6	0	2	0	0	0	1
27	0	1	2	2	2	0	7	0	1	0	0	0	0
28	0	1	0	0	2	0	8	1	1	0	0	1	0
29	1	1	1	0	1	1	9	0	1	1	1	2	0
30	1	3	2	0	1	2	10	1	1	0	0	1	0
31	1	1	1	1	1	0	11	1	0	0	0	1	0
32	1	0	1	1	1	0	12	0	0	0	0	0	1
33	1	0	2	0	1	1	13	1	0	0	0	1	1
34	1	1	0	0	2	0	14	1	1	1	0	1	1
35	1	1	3	0	1	0	15	0	0	2	1	1	0
36	0	1	1	0	2	0	16	0	2	1	0	3	0
37	0	1	1	1	1	1	17	0	3	1	0	1	0
38	1	2	1	0	2	1	18	1	2	1	0	1	0
39	1	1	3	1	1	0	19	0	2	1	0	2	1
40	1	1	0	0	0	1	20	0	1	0	0	0	0
41	0	0	0	1	0	0	C 1	0	0	1	0	1	0
42	0	1	1	0	2	0	2	0	0	1	1	1	0
43	0	2	0	1	1	2							
44	0	0	3	0	1	0							
Total								23	48	53	21	59	19 = 243

gangskrebs) and 17 cholangiocarcinoma associated with hepatomas among their 88 tumor livers. Orr's (1940) finding of many complexities of tumor kinds does not clarify the point, whether a single mass showed a mixture of patterns or different lesions in a liver represented several different histologic patterns.

Mulay and Firminger gave, in 1952, a clear cut classification of hepatoma,

cholangioma and cholangiohepatoma. The last entity is especially significant, so far as it means complexture of hepatoma and cholangioma which might possibly be separated by serial transplantation as Morris did. They admit two different sources of cancerous epithelium in experimental rat liver, which are reflected in histochemistory of established cancers. McManus' PAS stain and demonstration of alkaline phosphatase were suggested as especially useful in differentiating hepatoma and cholangioma.

In this study we classified the tumors obtained arbitarily in 6 categories. Corresponding terms of the previous authors are tabulated (Table 2). Our classification is based mainly on structural patterns. PAS-stain was found not very helpful, contrary to the opinion of Firminger and Mulay.

(3) Collision of tumors:

Most of macroscopic nodules are separated from each other by such benign structures and lesions as atrophic liver tissue, cholangiofibrosis and bundles of collagen fibers. The interlacing tissues may be at one time very broad and at others fairly narrow.

Macroscopically observable collision of two different tumors was only rarely seen. Some of grossly simple nodules represented histological pictures interpretable as being made up of two independent tumors colliding upon each other.

Not all of grossly dumb-bell shaped or hour-glass lesions, on one cut surface, represented histological duality. On the other hand they were, in the majority, main tumors and daughter nodules of the same histological category.

Some of such tumors might have been separate primary malignancies, coming into collision against each other, but many were of so similar histologic pattern that they were discarded as not suitable for this particular study.

Certain difficulties and subjectivity should have been overcome in the correct interpretation of the colliding histology. It has been our experience that a single homologous tumor may exhibit far reaching pleomorphism in itself, for example, ranging from a typical trabecular hepatoma to a hepatoma with cystopapillary glandular pattern (genotypically identical but phenotypicaly different). Each tumor was cut serially or stepwise to follow gradual transition from one type to another. Representative extremities of variation of one tumor are shown in Figures 1–4.

Disseminated areas of higher atypism and density of nuclear population were sometimes encountered without any variation in cytoplasmic or architectural pattern. Such actively proliferating centers often displace surrounding tumor tissues to simulate a colliding malignancy even in a single neoplasma.

Discrete collison are seen only in the following few cases:

A: (R 47–6) A large-celled solid hepatoma occupied the major portion of the periphery of the 5th lobe to encircle a rounded mass of small celled solid

Table 2. Classification of hepatic tumors induced in rat. Comparison of terms used by the authors and previous workers.

	Authors' Classification	Yoshida & Sasaki (1935)	Maruya (1939)	Orr (1940)	Edward (1941)	Opie (1944)	Richardson et al. (1951)	Mulay & Firminger (1952)
Hepatic cell origin	Solid Hepatoma (S.H.) (small celled) (large celled)	Atyp. H.	Solid Alveoler	C	II ?	(T.H.?)	Malignant hepatoma small celled large celled	Undiff.
	Trabecular Hepatoma (T.H.)	Typ. H.	Trab.	C	(I (II	T.H.		Hep.
	Adeno-hepatoma (A.H.)	Atyp. H.	Adenoca.	C C+B C+A ?	I	A.H.	Adenoca. (liver cell type)	Hep.
	Hepatoma with tubulo-glandular pattern.	Typ. H. Atyp. H.	Atyp. H. mixed	C C+A C+B ? C+A+B	I	(A.H.)	(Anaplastic ca.)	Cholang. hep.
Bile duct origin	Cholangiocarcinoma (CHC)	G.G.K.	(−)	Adc.	Ad.	Chol.	Adenoca. (bile duct type)	Chol.
	Sarcoma (SA)							

hepatoma in the central region. Relatively sharp delineation of the two tumors with indentation of the bordering surface, is clearly seen in low magnification (Fig. 5).

In high power view, they are separated in some parts by a thin capsule of collagenous fibers of 20–30 micra thickness, but in the area of indentation small and large cell tumor components are intermingled to form a zone, 100–200 micra thick, in which mitotic figures are seen exclusively in the larger cells, which grow at the cost of the smaller cells, the latter showing such signs of degeneration as pycnosis, karyorhexis and compression atrophy. Invasion of the large celled components into the scanty stroma of the small-celled hepatoma is also observed (Fig. 6).

B. (C 11–6): A clear cut dumb-bell shaped collision of two tumors, one a large celled solid hepatoma, 250 micra in diameter, and the other a small celled solid hepatoma of 100 micra, is seen (Fig. 7) in the central part of the 5th lobe. Contact surface of the two is rather small. In low power their border is fairly sharp. Higher magnification reveals beginning infiltration of the large celled part by the smaller tumor cells. Some nuclei of the former are falling into karyolysis. The paralled capillary septa appear to represent former territory of the large celled hepatoma (Fig. 8).

C. (R 43–Z): The 2nd lobe of this rat's liver contains three coalesced lesions. The largest one is a cholangiocarcinoma and the smallest an adjacent daughter nodule of the former (Fig. 9). To the other side of the lobe, a third mass, a solid hepatoma, is seen contacting with the other two nodules. The largest nodules is roughly ovoid, with a stretching arm embracing the solid hepatoma and also with a strand of tumor elements protruding into the latter. Degeneration on the side of the solid hepatoma, assosiated with displacement and replacement by the cholangiocarcinoma cells, is noticeable in higher magnification (Fig. 10).

D. (R 30–1): The 1st lobe is almost entirely occupied by three large masses, filling in the entire thickness of it. Two nodules on the right are hepatomas of the histology, ranging from solid to trabecular hepatoma pattern. The nodule to the far left, is a typical cholangiocarcinoma. Bordering capsule consists of a thin layer of collagenous tissue, which is perforated in the center, by a penetrating mass of the cholangiocarcinoma.

Degeneration, displacement and replacement of the hepatoma tissue caused by the invading cholangiocarcinoma are associated with local hemorrhage, hemosiderosis and necrosis. Mitotic figures are definitely more frequently encountered within the latter elements (Figs. 11 and 12).

E. (R 30–3): The third lobe of the same rat's liver shows similar conditions as in the 1st lobe. Only the colliding tumors are slightly smaller in size, while the dividing surface of a trabecular hepatoma and a cholangiocarcinoma shows

marked waviness. Both the hepatoma and cholangiocarcinoma are rather low in atypism and cellularity. They appear to be in balance. Intermingling of tumor cells are seen in a thin layer of their borderline (Figs. 13 and 14).

F. (C 19–Z): A grossly simple ovoid mass occupies the 3rd lobe. A typical trabecular hepatoma, which shows slight tendency to form glandular structures in the other parts, as revealed by serial sectioning, complies the dimensional majority. At one extremity there is a small area of cholangiocarcinoma, accompanied by neighboring cholangiofibrosis (Fig. 15). At the borderline, compression exerted by the hepatoma on the cholangiocarcinomatous tubular structures is obvious (Fig. 16).

G. (C 6–Z 2): Three tumor masses are seen in the 4th lobe: a small independent adenohepatoma, and a trabecular hepatoma with occasional glandular structures in the peripheral part of the lobe, surround a third tumor, a cholangiocarcinoma associated with cholangiofibrosis in the central partion of the lobe. Collision has occured between the two latter tumors (Figs. 17 and 18).

Discussion

In the first two cases colliding tumors were hepatomas of different patterns, and in the last six collision has occurred between hepatomas and cholangiocarcinomas. In some, the two participants in the collision are of similar size (D & E). Certain state of balance is some times seen (B & E). But in the majority one is apparently overwhelming the other (A, C, D, F, G & H). The final stage ot collision is suggested in G. The field of combat has been occupied by the more active participant in the collision.

Histological analogy of this tumor-vs.-tumor collision is demonstrable in the interrelationship of a cancer vs. a normal liver tissue, or a cancer vs. a benign adenoma or cholangiofibrosis. In such cases the sequels in the field of collision are unequivocal from the first. Displacement, replacement, infiltration, degeneration of the parenchyma, especially of the passive tissue, associated with certain circulatory distrubances *in loco* are common to all kinds of collision seen.

Mulay and Firminger (1952) have suggested possibility of collision of tumors, in interpreting a rather sharp delineation of two different histological patterns in a cancerous nodule. Lack of study with serial sections prevented them from drawing a conclusion.

In one case (C 12–Z 2) collision between a trabecular hepatoma and a sarcoma was seen, in which the sarcomatous components very diffusely infiltrated the carcinomatous area. Finding on such carcino-sarcomas in our experimental series will be reported elsewhere.

According to Mulay & Firminger, Moris has recently demonstrated that in a colliding tumor, separation of the two components had been possible by way of

serial transplantation of a grossly single tumor nodule. Interpretation of Moris's findings appears not to be simple.

In the present studies serial transplantation was tried from 27 cancerous livers. For the purpose of our studies, this procedure appeared to us not very helpful from the first. Resulted serial transplants showed always one or the other histological pattern, although the transplantation was followed up to the thirtyth generation.

In view of the above results, we assume that it may not be premature to draw a conclusion that, at least in certain cases, collision of two heterologous tumors occurs in a limited field of cancerization, as each of the independent foci grows further. Apparently the less actively proliferating participant in collision will be overcome by the more active one and will disappear from the field. The field becomes occupied by one single tumor. Thus heterologous duality of the first gross nodule turns into a hemogenity. It resembles the actuality in the human society: survival of the more powerful one.

If we consider the ealier periods of cancerization in a more restricted field, elimination of tumors of minor proliferative potentiality by more active one is quite reasonably suggested. It may occur also *in statu nascendi* of tumors. Generalization of this thesis also suggests elimination, rather than apposition in the growth of a single gross tumor. If it were true, mutative genesis of a cancer could be more reasonably accepted in the pathology of tumors.

Summary

1. Some examples of colliding tumors in experimental DAB rat liver cancers are presented.

2. All the tumors encountered in the experiment were examined histologically and classified. Their ranges of structural variation were determinated.

3. Histology of collision between two cancers, or of invasion of a tumor by a second and more potent tumor was described. Passive behavior of the invaded tumor was analogous to normal tissue under infiltration by a cancer.

4. End result of collision would most likely lead to occupation of the field by one tumor, superior to the other in proliferative potency.

5. Development of a cancer in a field of cancerization is by way of elimination of minor cancers *in statu nascendi* by a potent cancer, and not in the mode of appositional growth.

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要　旨

実験的多中心性ネズミ肝癌における初期癌巣間の相互交渉について

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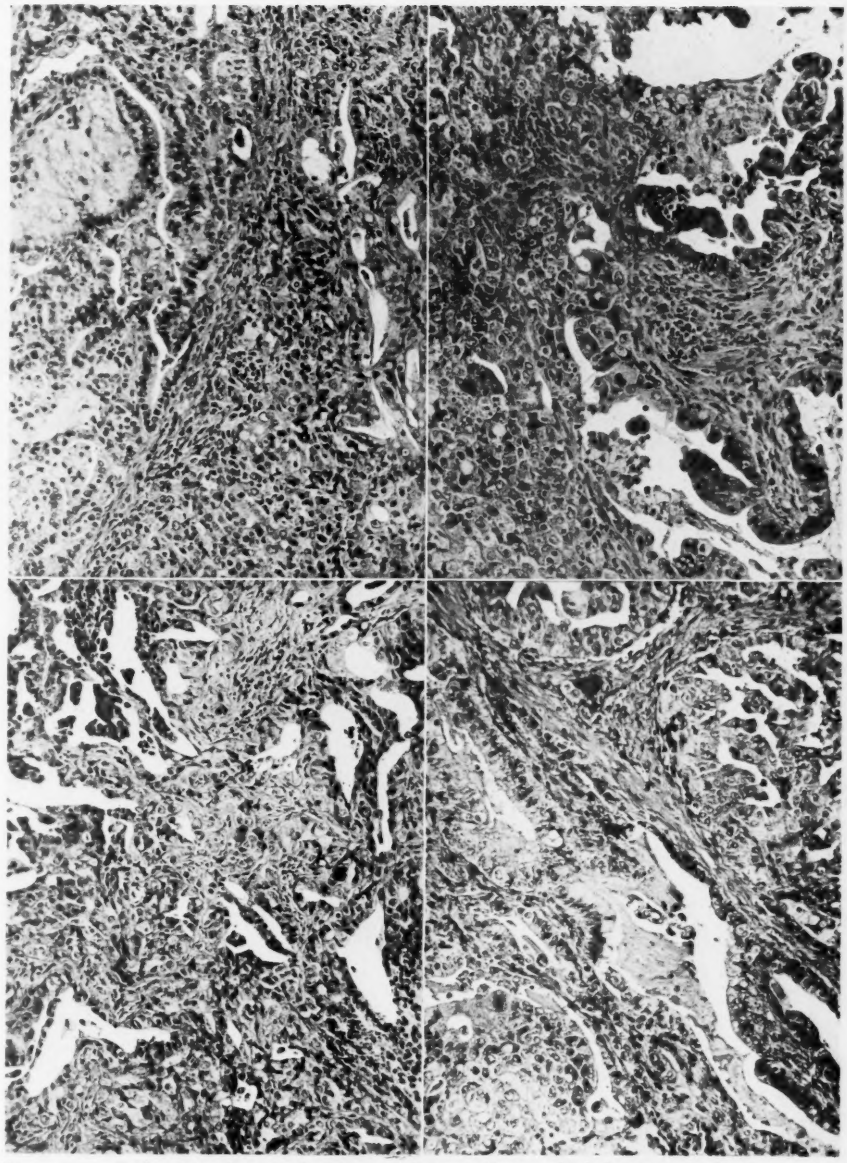
ある発癌の場においては，発癌は多中心性に行われるにもかかわらず，通常発達した癌は一個の結節を形成し，形態学的特徴，移植上の態度から単一腫瘍と看做しうる場合が多い。著者らは多中心性に生じた初期癌が発達した単一の癌塊として認められるに至る過程において，二種以上の癌巣間の衝突並びに一つの癌による他の癌の圧迫を，dimethyl-aminoazobenzene ネズミ肝癌実験によって実証することができた。多中心性発癌が単一癌塊に発達するには，優勢な一種の癌による劣勢の癌の征服が行われる。優勢な癌による劣勢な癌の同化 (Assimilation) なる機転は証明されなかった。



Figs. 1–4. Examples of several patterns of tumor tissue which forms a single gross mass. Variation in a single tumor is fairly wide-ranged. Even transition from the trabecular hepatomatous pattern to the papillary cholangiocarcinomatous type is observed. Gradual transformation from a type to another is demonstrated in these high power figures.

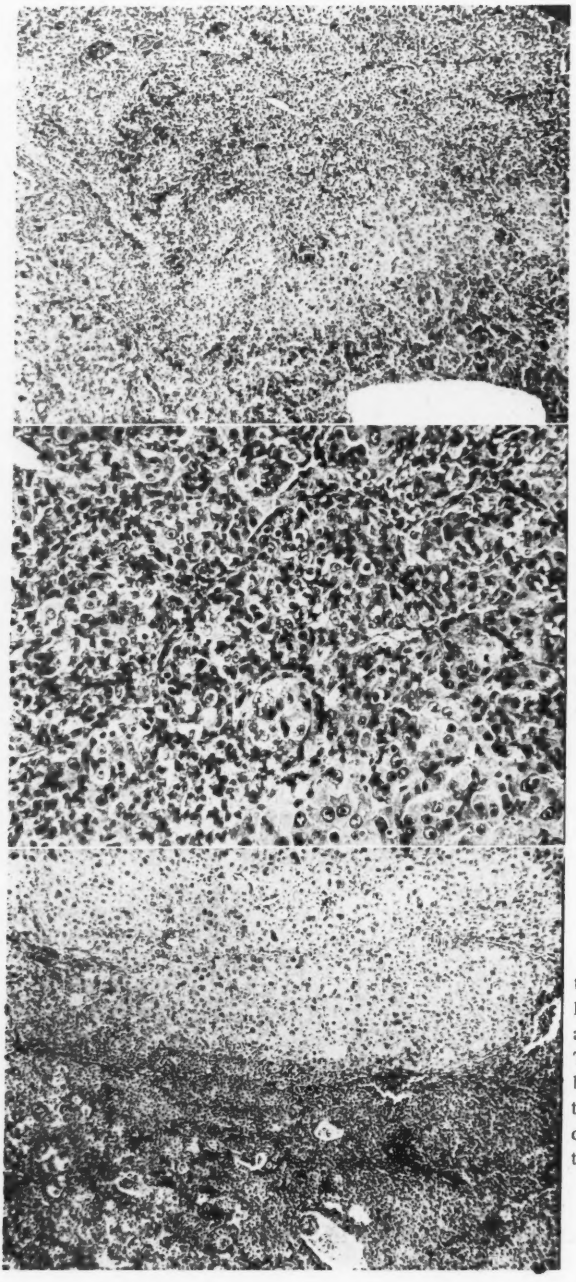


Fig. 5. A low power view of collision of a large celled solid hepatoma (upper) upon a smalled celled variety of solid hepatoma (below). A clear indented line between the two tumors is observed. (R-47-6).

Fig. 6. A high power view of the middle left hand field of the Fig. 5. Degenerative changes on the part of the small celled hepatoma in front of the invading large celled hepatoma (below) are seen along the indented separating line. (R-47-6).

Fig. 7. A low power view of the colliding front between a large celled solid hepatoma and a smalled celled one. (C-11-6). The limiting line is fairly clear, but on left upper hand infiltrative in vasion of the small celled components into the large celled tumor is observed.

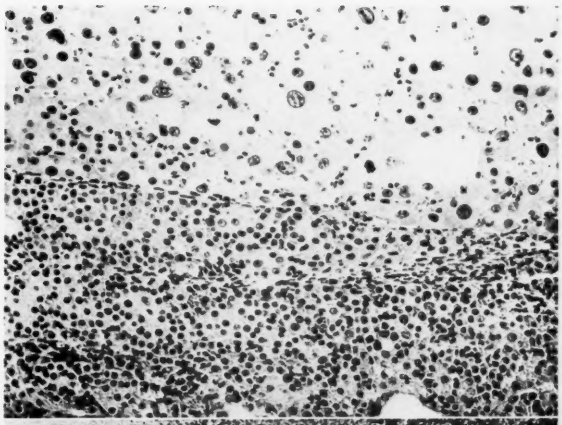


Fig. 8. Higher magnification of the central field of Fig. 7 to show the details. (C–11–6). There is no transition between the two kinds of tumor.

Fig. 9. General pattern of the largest nodule in the 2nd lobe of R–43. Note the irregular papillary pattern with disseminated foci of necrosis. A solid structure is not seen.

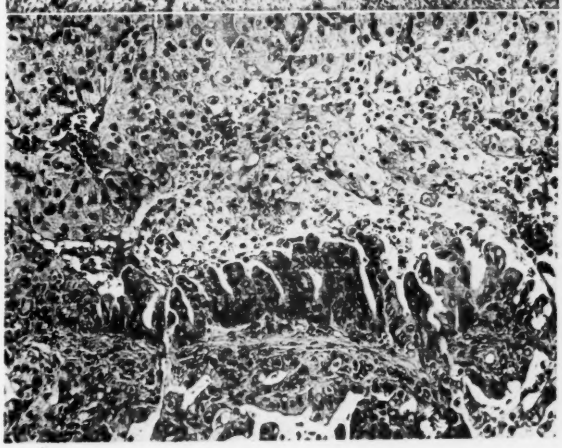
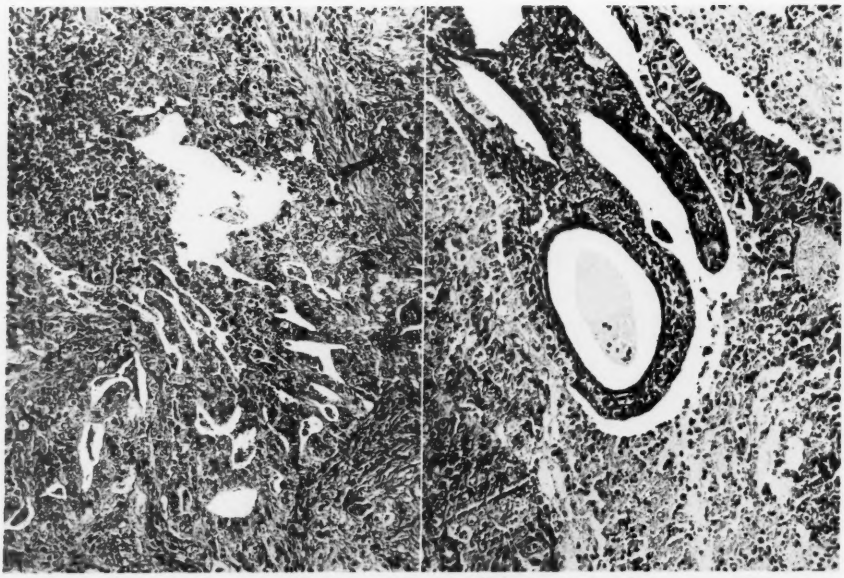
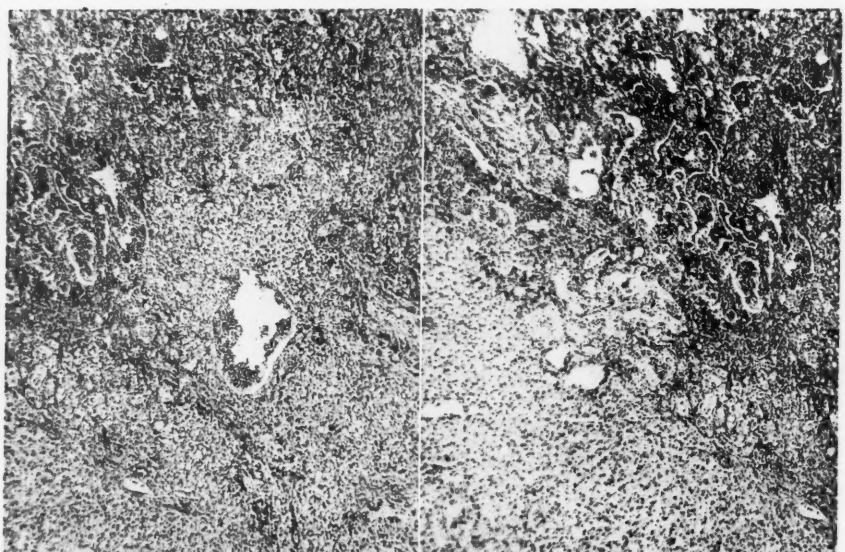


Fig. 10. A high power view of the colliding front of the cholangiocarcinoma of papillary pattern upon a solid hepatoma nodule adjacent to the former. (R–43–Z).



Figs. 11. & 12. Higher magnification of the invading front of a cholangiocarcinoma into a trabecular hepatoma. Note the compression on the latter associated with degeneration and local hemorrhage. (R-30-1).



Figs. 13. & 14. Low power views of another example (R-30-3) of invasion of a regular trabecular hepatoma by a tubular cholangiocarcinoma. Note the indentation and complexity along the limiting line between the two tumors.

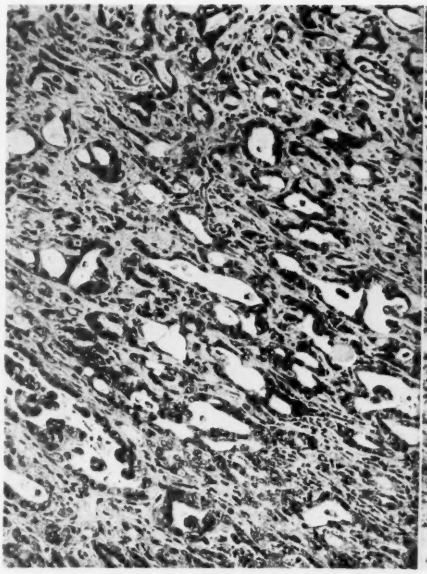


Fig. 15. A typical tubular cholangiocarcinoma arising in a focus of cholangiofibrosis. (C-19 Z).

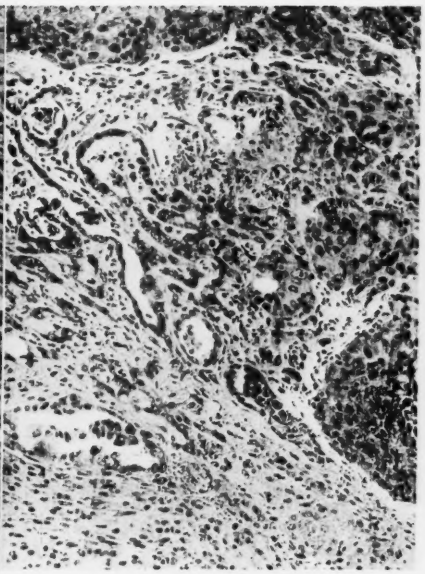
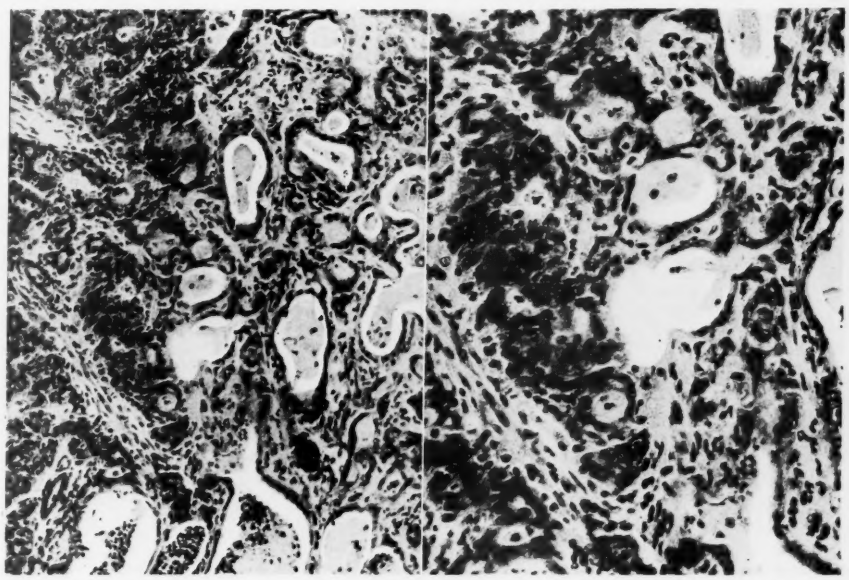


Fig. 16. A high power view of the contact surface of two tumor nodules in C-19 rat liver. The rignt upper hand field is occupied by a typical tabecular hepatoma. The left hand tumor is the extension of a tubular cholangiocarcinoma shown in Fig. 15 above. Note the hepatoma opening a tubule of the cholangioma and necrosis.



Figs. 17. & 18. A low and a high power view of the colliding front between a trabecular hepatoma and a cholangiocarcinoma. Note the degeneration of the cancerous epithelium of the tubular cholangiocarcinoma in front of the hepatoma. (C-6 Z 2).

[GANN, Vol. 45; December, 1954]

ON MESENCHYMAL REACTION OF RAT LIVER IN AZO-DYE CARCINOGENESIS, ESPECIALLY ON THE FORMATION OF CARTILAGE. SPECIAL CONSIDERATION ON THE MIXED TUMOR OF THE LIVER. (With Plates XV—XVII)

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In the vast series of azo-dye rat hepatoma experiments since Yoshida and Sakaki (1935), Kinoshita (1937) first stressed importance of initial mesenchymal proliferation in the periportal area in the course of formation of cholangiomas by DAB., Maruya (1941) saw occasional myxomatous structures, Edwards and White (1941) myxomatous structure with scattered islands of cartilage, and Mulay & Firminger (1952) bone and cartilage formation. Richardson et al. (1951) found among 57 tumor bearing rats fed 3'-methy-4-dimethyl-aminoazobenzene sarcoma 5 times and bone and cartilage formation 4 times. No detailed description or discussion has been attempted concerning mesenchymal reaction seen in the liver of azo-dye fed rats.

Material for this study consists of 8 rat liver tumors, in three series of experiments with DAB-feeding as listed below. One additional tumor is a second generation transplant of a DAB-hepatoma. Details of the experimental series are reported elsewhere.

Table 1. List of Materials

	Rat (diet)	Lobe	Size	Carcinoma	Mesenchyma			Days of Exp.	DAB mg.
					Bone	Cartilage	Mesenchyme		
A	R-39 (Rice)	2nd	1.0 × 1.0 × 1.0	Trabecular hepatoma with glands (##) Mucin (#)	+ (osteoid)	##	# cellular, stellate	182	566
B	R-35 (Rice)	5th	5.0 × 5.0 × 5.0	Cholangio-carcinoma Muc. (#)—	—	+	# not very cellular, stellate	173	673
C	R-28 (Rice)	2nd	4.5 × 4.0 × 2.5	Cholangioca. +Ad. hepa. glandular Muc. (##)	—	#	# not very cellular, stellate	156	541

D	R-37 (Rice)	4th	5.0 × 1.0 × 4.0	Cholangioma Muc. (±)—	—	+	± cellular	175	592
E	R-33 (Rice) (§Transplant)	5th		Solid hepa. + glandular	—	±	± cellular	166	566
F	C-12 (Crisco)	4th		trabecular hepatoma Glandular Cholangio. ca.	—	+	+ not very cellular, stellate	378	832
G	C-13 (Crisco)	2nd	2.0 × 2.5 × 2.2	Solid hepatoma Trab. hepa. + glandular	—	+	+ cellular	387	832
H	R-38 (Rice)	4th		Solid hepatoma + glandular	—	—	± (Ca-Sa.)	178	590

Findings

(A) R-39-Z: An 1 cm sized tumor in the middle of the second lobe of the liver was proved to be a composite mass, mainly of trabecular hepatoma with development of tubular structures, and a broad sheet of highly cellular mesenchymal tissue, well intermingled with the former and playing the part of the stroma for the carcinomatous epithelium. Scattered in the mesenchymal mass multiple islands of hyaline certilage are present. Some strands of osteoid tissue are also seen, usually imperfectly surrounding the cartilaginous islands. Concentric arrangement of flattered spindle cells around cartilage shows definite transition to undifferentiated mesenchyme of the remaining parts, consisting of loose syncytium of stellated cells. Osteoid ground substance is deposited between those stellated cell in a shape of strands. Mitotic figures are fairly frequent. There is no cellular atypism (Fig. 3).

Boundary between the epithelial and mesenchymal elements is clear cut, and although imperfect, some basement membrane-like structures are seen. Direct contact or transition of the epithelium and cartilage has never been found. Both epithelial mucin and cartilaginous ground substance show positive mucicarmine stain, and metachromasia to toluidin blue at pH 3.6. McManus PAS stain reveals definite difference between the two.

(B) R-35-9: An irregularly ovoid, soft and grey-white mass of 5.0 cm diameter occupies the 5th lobe of the liver. It is mainly made up of fairly well

differentiated adenocarcinoma of cholangiomatous type. Some parts are solid, but marked honeycombed structures prevail in the other parts. Stromal tissues are unique. It consists of an edematous loose mass of well differentiated stellate cells with pale eosinophilic cytoplasms. Spindle cells are rarely encountered. Capillaries are notably distended. A few islands of hyalin cartilage are seen scattered in this mesenchymal mass. Owing to paucity in cellularity of the loose counective tissue, such cartilaginous tissue appears to exist without transitionary zones. Some islands come almost in contact with cancerous tubules (Fig. 1).

In contrast to the previous case, here we see frequently destruction of carcinomatous tubules, which spill out mucinous content into the subjacent mesenchyme. Transition between cartilaginous ground substance and spilled mucin has never been seen.

(C) R-28-3: The 2nd lobe had 4.5×4.0×2.5 cm ovoid grey-white mass, which is made up mainly of carcinomas, a cholangiocarcinoma and an adenohepatoma apparently colliding upon each other. Advanced cholangiofibrosis is present in close relation to the cholangiocarcinoma. Stroma of the adenohepatoma is narrow and not remarkable, while that of the cholangiocarcinoma is fairly broad and consists of characteristic structures. Fibers directly subjacent to the cholangiocarcinomatous epithelium are definitely collagenous with long sharp spindle cells. The cores of the stroma are loose stellate cells with multiple tiny rounded masses of hyaline cartilage. Transitions between the collagenous part and loose stellate mass, and between the latter and cartilaginous islands, are obvious. Some carcinomatous tubules are distended, some are collapsed and degenerated, with their mucinous contents spilling out into the subjacent mesenchymal mass. No reactive response or organizing process is seen. Cartilages are quite independent from this spilled mucin (Fig. 2).

(D) R-37-2: A soft grey-white mass of 5.0×6.0×4.0 cm, with occasional hemorrhagic-necrotic foci, occupied almost the entire 4th liver lobe. It consists of cholangioma with remarkably distended glandular structures, which spill out mucinous mass into the subjacent stroma. In the rather broad sheats of loose stellate cell mesenchyme, many large islands of hyaline cartilage are scattered. High cellularity of the undifferentiated mesenchyme and well differentiated cartilaginous structures are quite similar to those in the first example (Fig. 5).

(E) R-33: A small (0.5 cm), rather firm, grey-white mass from the 5th lobe, which was resected on the 166th day of experiment for serial transplantation, consists of a solid hepatoma with partly dilated glandular structures. Stromas are narrow and non-remarkable, except a few sparsely scattered, minute cartilaginous nodules. Although not fully differentiated, they have every characteris-

tic of hyaline cartilage with metachromatic hemogenous ground substance embedding ballooned chondros. Usual young mesenchymal masses, intervening between those nodules and carcinomatous epithelium are almost entirely lacking. The nodules often appear as if they were inserted into the narrow strands of stroma artificially. Spilling out of mucin is not noted.

(E′) The second generation of the tumor E, submitted to a serial transplantation to peritoneal cavity of stock rats, showed, on the 14th day of transplantation, similar finding as in E. Only the cartilaginous masses are somewhat larger than those observed in the original tumor. Stellate mesenchymal tissues are just noticeable between the hyaline cartilages and carcinomatous epithelium of a highly cellulated solid hepatoma showing no glandular structures in it. Some cartilaginous masses have apparently undergone degeneration, granulated, basophilic and necrobiotic (Fig. 4).

Further serial transplants up to the 30th generation have so far revealed no such cartilaginous mass.

(F) C-12-Z 4: The 4th lobe contained a trabecular hepatoma with glandular structures and a cholanginoma associated with cholangiofibrosis. A few small islands of hyaline cartilage were seen scattered in broad sheat of relatively mature stellate cell mesenchyme. General pattern is quite similar to that of (B) (Fig. 6).

(G) C-13-2: Multicystic dilated structures of cholangiocarcinoma, measuring 2.0×2.5×2.0 cm in greates dimensions, occupies the 2nd lobe. Hemorrhagic necrosis has occurred rather extensively. Only a single minute island of cartilage has been found in step sections. Undifferentiated mesenchyme resembles with that of the previous case.

(H) R-38: The 4th lobe is entirely replaced by a solid hepatoma with a part of glandular structures at one side, where the interstitial tissues are remarkably broad and consist of dense sheets of middle sized stellate cells. High atypism, hyperchromatism and abundant mitosis of their nuclei, associated with polymorphism of cytoplasm, occasionally forming small giant cells, strongly suggest the stromal mass being malignant. No transition is observed between the carcinomatous epithelium and the stromal cells. Adjacent liver capsule is infiltrated only by the atypical stromal cells. (Fig. 7).

Summary and Discussion

1. Types of mesenchymal proliferation above are as follows:

a) Young edematous mesenchyme, consisting of loose mass of stellate cells or of fat spindle cells of non-atypical appearance, around cancerous alveoli or cartilage. It can be at times very highly cellular with crowded fat spindle cells, which have basophilic cytoplasm, but also can be made up of sparsely disseminated

rather acidophilic cells, anastomosing each other. Lymphocytes or other free cells are only very rarely seen (Fig. 1).

Of note is the sharp delineation of the proliferating immature mesenchyme against adult fibrous interstitium, composing normal liver parts, oridinary stroma of hepatic cancers or cholangiofibrosis.

b) Islands of hyaline cartilage, regularly surrounded by loose young mesenchyme above. Although they can be quite close to other structures, they are never seen exposed. They never show atypism, either of nuclei or cytoplasm. Their ground substance is that of regular hyaline cartilage (Fig. 1).

c) Fragments of osteoid tissue embedded in the young mesenchyme, and located in the midportion between an island of hyaline cartilage and a cancerous alveolus. They lack of any kind of malignant atypism, or of high proliferative activity. Calcification is very limited.

Such findings as indicate conversion of cartilage into osteoid or bone were never encountered. Membraneous character of the bone is apparent. Sometimes an arteriole is seen in the center or vicinity of osteoid formation.

d) High atypism of the mesenchymal elements, showing invasive growth into the hepatic capsule and diaphragm, and with suggested focal destruction of coexsitent cagcinomatous tissue, was found only in the last example (Fig. 7 and 8)

2. Epithelial components and mesenchyme:

a) As has been stated the loose mesenchymal tissues are very intimate with the cancerous epithelium. They act precisely as a host stroma of the carcinoma, playing the role of primary stroma (Yoshida), which supplies both nourishment and stand point to the malignant epithelioma. They never appear to be invaded, lysed or amputated by the cancer. Cancer and mesenchyme make up an organoid structure. Necrosis and degenerative process are extremely rare. Degeneration of carcinomatous tubules or cystic glands appears to occur from other causes. Mucinous contents may be spilled out or left in the mesenchyme, without causing remarkable reactive change in the latter. Myxomatous transformation, as observed by Kinoshita, was unusual in our series. We could not confirm Kinoshita's finding that the proliferated mesenchymal mass exerts pressure upon the parenchyma, either benign or malignant. Cartilaginous element is usually situated in the center of a mesenchymal stand, showing definite transition between the two kinds of tissue.

b) Pattern of the epithelial elements does not vary very much in relation to the mesenchymal reaction. Liver parencyma, bile ducts, hepatic adenoma and cholangiofibrosis have constantly connective tissues of adult type as their interstitial tissues.

Carcinomas associated with this type of mesenchymal proliferation are pre-

dominantly cholangiocarcinoma with cyst-like dilated spaces or hepatoma with glandular pattern. Close relationship between the carcinomatous epithelium which lines glandular spaces and the mesenchymal reaction is of note. Many of such tumor types are associated with cholangiofibrosis. It is also note-worthy that focal squamous metaplasia was seen several times in the atypical columnar epithelium adjacent to islands of cartilage (Figs. 4, 5 and 6).

Kinoshita (1937) describes in detail proliferation of connective tissues in the early histogenesis of cholangioma. Sometimes, in his experimental series, proliferation of stellated cells were so active that, in association with degenerative changes on the part of epithelial elements, myxomatous structures occurred. He also got a "hepatoma combined with reticulosarcoma." Fig. 57 of Kinoshita almost exactly reduplicates our findings in No. A, D, E, G with marked cellular proliferation of undifferentiated mesenchymal stellate cells. Neither nuclear or cytoplasmic atypism is conspicuous.

Coordinate effects of DAB on parenchyma cell of the liver on one hand, and on the stromal tissue, including endothelial cells, reticulum cells and fibroblasts have already been pointed out, also by Kinoshita.

Incidence of cartilaginous structures was considerably high in our series, as compared with that of the previous workers. 13.7% of 51 tumor bearing rats, and 0.036% of 223 gross tumors found in the rat liver contained such structures.

A positive relationship between the kind of basic diet and incidence of hyperplastic mesenchymal reaction was not confirmed, although the group R (rice diet) showed highest incidence of 14.7 % and none of the group B (butter diet) had cartilage containing tumor.

Chemical variation of carcinogenic amino-azo dyes appears so far to be of little consequence in inducing mesenchymal reaction in discussion.

Richardson et al. stated that bone and cartilage appeared after 21 weeks of experiment. In our present series the peculiar reactions were observed in the rats kept for rather long period, ranging from 156 to 378 days.

Rats apparently tend to produce cartilage in liver under experimental conditions. Kinoshita has got stellate cell proliferation in fowls, Andervont and Dunn hemangioendothelioma in mice, and Nelson and Geoffrey (1953) some mesenchymal proliferation in dogs after feeding amino-azo dye, but no cartilage. Bullock and Curtis (1925) found four cartilage containing tumors among 1,400 rats infested with Cysticercus fasciolaris. Two of them contained hyaline cartilage and two contained both cartilage and osteoid tissue or bone. They were mixed cell sarcomas containing islands of cartilage, a chondrosarcoma, an osteochondrosarcoma and an osteoid chondroma. Another case of cysticercus tumor of rat, with a chondrosarcoma and a carcinosarcoma containing chondromatous components, was added by the same authors (1928). They used a strain

of Copenhaagen rats. Other stock rats have up to present produced (1951) cysticercus sarcoma rather infrequently.

Foulds (1937) reported a strain of transplantable oviduct carcinoma in domestic fowls which on serial transplantation produced bone and cartilage. His interpretation was that the carcinoma induced host stroma to form such structures. Undifferentiated mesenchymal reaction occurred always antecedent to such special structures. Andervont & Dunn's findings (1952) on serial transplantation of induced and spontaneous hepatomas in homologous mice was very remarkable. They saw hemangioendotheliomas and reticulum cell sarcomas arose from the peripheries of transplants. Hyperplasia of surrounding mesenchymal tissue appeared to precede such induced heterologous malignancies. Transplanted hepatoma disappeared as the second malignancy grew to take its place.

14 hepatic tumors in this series were excised by laparatomy from the primary hosts, and transplanted into other albino rats of the same stock, serially. Only 4 took in the first generation, of which one was followed up to the 30 th generation. Cartilaginous mass was observed only in the second generation of transplantation and failed to reappear in the following generation to the end. The cartilage appeared more mature in the transplant than in the original liver tumor and somewhat degenerated, suggesting that it was not a autochthnous proliferation.

There have been many report since Apolant & Ehrlich (1906) concerning transformation of an animal carcinoma into a sarcoma during serial transplantation. Sanford et al. (1952) have obtained sarcoma by implantation of serially cultured carcinomas of liver, breast and thyroid. Toolan & Kidd (1950) saw a mammary carcinoma transforming into a sarcoma in an immunized animal. Such unexpected findings of transformation of epithelial tumors into mesenchymal ones, although they can be interpreted otherwise, might suggest epithelial character of the "mesenchymal" hyperplasia in appearance. However, in our experiments, thorough examination failed to reveal any transition of the carcinomatous elements and stromal hyperplasia.

Two possibilities are suggested for the causation of this peculiar mesenchymal proliferation with cartilaginous differentiation. This may be either a primary reaction of the hepatic interstitium to the carcinogenic substance, or a secondary induction in the stroma by the epithelial malignancy, first produced by the carcinogen. In view of rather rare occurrence, close histological relation to the epithelial components and long latency, the latter interpretation seems to be more acceptable.

On the other hand, knowledge has been accumulated concerning mixed tumor of human liver. Among many reports, only a few are acceptable as tridermal neoplasma, instead, majority of them appear to be more adequately explained

on the basis of bidermal hyperplasia: hyperplasia of entodermal and mesodermal components only. In many cases, epithelial hyperplasia is far predominant over the mesenchymal one, which may actually be due to secondary induction exerted by the epithelial tumor (Someya, 1951, directed by one of the authors).

Resumé

1. Incidence of peculiar mesenchymal hyperplasia with formation of cartilage was 13.7 % of all tumor bearing rats fed with DAB.
2. Histological details were described.
3. Its histogenesis was discussed. Theory of secondary induction by DAB induced cholangiocarcinoma and glandular hepatoma was favored in its causation.
4. Histogenesis of mixed tumor in human liver may be interpreted also in this way.

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要　旨

癌原性アゾ色素によるネズミ肝の間葉性反応，特に軟骨形成及び肝の混合腫瘍の発生について

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木下 (1937) 以来，dimethyl-aminoazo-benzene ネズミ肝癌実験において，間葉性反応の著しいことが注目されてきたが，これについての詳細な記載は不足していた。著者らは，米及び高脂肪食を基本食餌として行った同実験について，間葉性反応を分析記載し，ことに軟骨島の出現と，間葉性反応一般並びに肝癌上皮成分との相関関係について論じた。軟骨島は肝癌 51 例中 8 例において見られ，一例では移植一代にも現われた。また一例の癌肉腫についても記載した。すべて上記の例に著しい間葉性増殖が認められ，幼若型から種々の段階の分化をも追求できた。軟骨島が，腺管形成のある癌組織に直接する間質に認め得られたことは，癌上皮の間葉分化誘導を示唆すると思われる。

Fig. 1. (R 35-9) The edematous mesenchyme with stellate cells. Note paucity in celluar population. Cartilage is seen in the center.

Fig. 2. (R 28) A young cartilage surrounded by fat spindle cells. On the right carcinomatous glands with mucinous secretion. Note that there is no transition between the cartilage and carcinomatous epithelium.

Fig. 3. (R 39-Z) Cartilage in a dense population of proliferating mesenchyme.

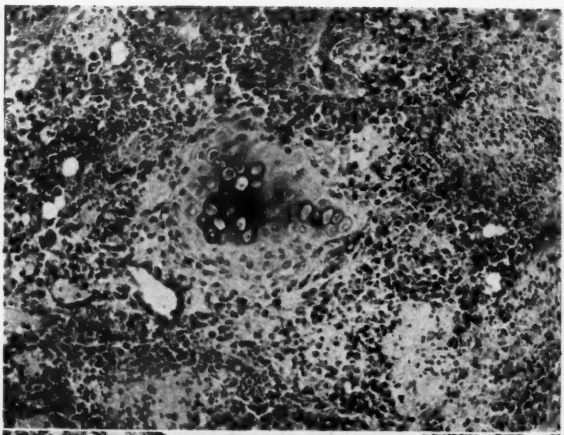

Fig. 4. (R-33E'-Transplant) Cartilage found in a second generation transplant of a cartilage containing hepatoma. Degenerative changes are present. 14th transplantation day.

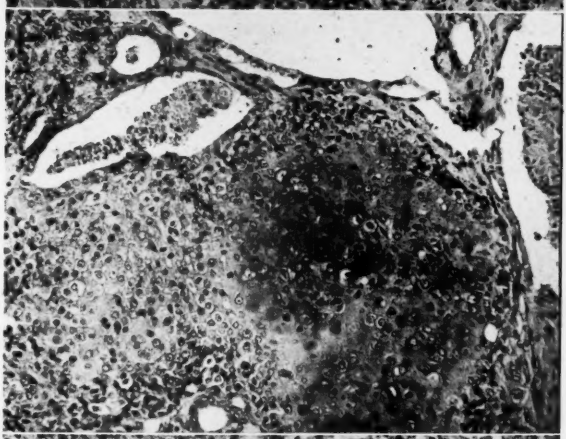

Fig. 5. (R 37-2 b) Relationship between glandular structures of the carcinoma and cartilage

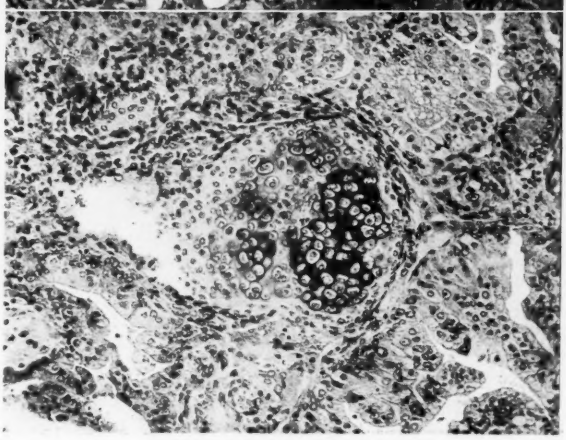

Fig. 6. (C 12-Z4) An abrupt appearance of a cartilageneous islet in parenchymatous carcinoma. Surrounding mesenchyme is remarkably thin.

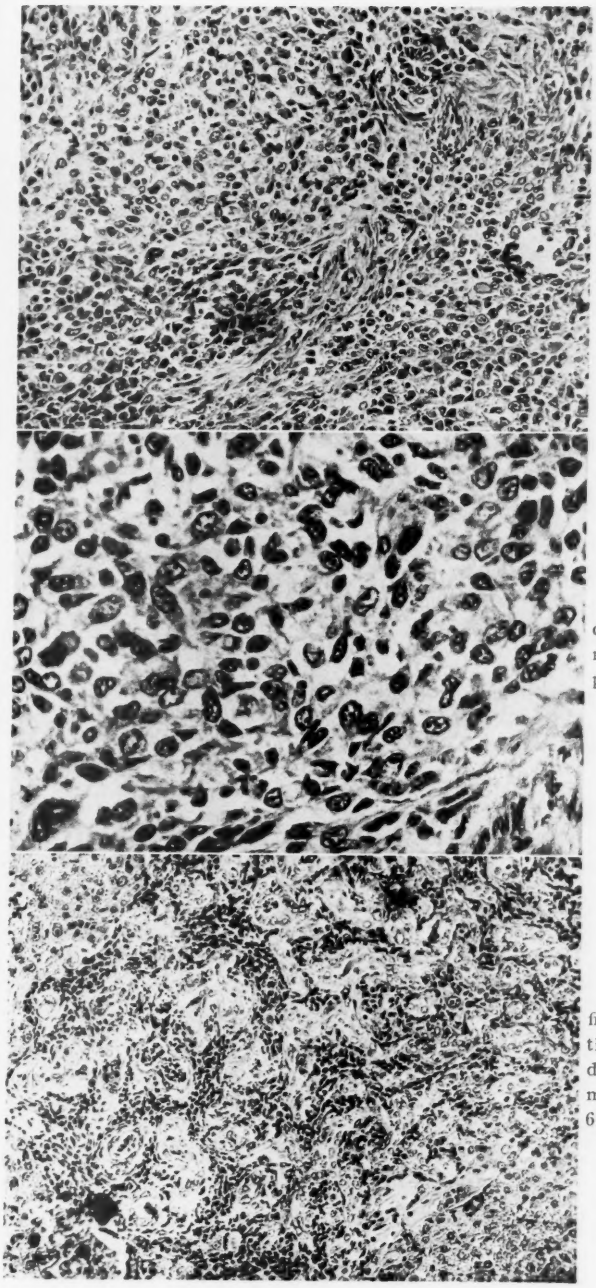

Fig. 7. (R 38-3) Sarcomatous proliferation of the mesenchymal components. On far right and below degenerated hepatoma cells.

Fig. 8. (R 38-3) Detail of celluar pattern in Fig. 7. Note marked atypism and polymorphism.

Fig. 9. (R 22-1) Cholangiofibrosis, mature connective tissue in this picture is quite different from the peculiar immature mesenchyme in Fig. 1.-6.

[GANN, Vol. 45; December, 1954]

STUDIES ON THE INTERRUPTION OF THE FERMENT SYSTEM OF GLUCOSE METABOLISM IN THE SARCOMA PRODUCING TISSUES. 2nd REPORT SUPPLEMENTALLY EVIDENCE OF THE SH-CONJUGATION HYPOTHESIS IN CARCINOGENESIS

(With Plates XVIII and XIX)

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INTRODUCTION

Ever since Nishiyama (1938) produced sarcoma experimentally by subcutaneously administering concentrated glucose came to be known as a carcinogen. Later, glycogen (Amano, 1943, Ito, 1944) lactose and fructose (Takizawa, 1938, 39) were found to evoke sarcomas. The altered sugar metabolism has long been considered, since the time of Warburg, as the main feature of the biochemistry of the neoplastic tissue. Glucose and glycogen as the carcinogens attract the particular attention, since they are the sugars physiologically intrinsic to human body. In the previous experiment (Report 1, Amano et al., 1950) the author tried to elucidate at what stage of the degradation such sugars are first involved in the chain of the carcinogenic mechanism. Namely, the author gave sodium fluoricum and monoiodoacetic acid—agents capable of interrupting glycolysis—together with glucose to the animals. It was thus possible to demonstrate that the stage responsible for the carcinogenesis was at the earlier stage of degradation (the stage before the level of triose). In this study, however, oaminoazotoluene, an accelerator, was applied to the site of the injection of glucose and monoiodoacetic acid following Nishiyama's method. Consequently, a question arose as to the existence or / and the mode of the interactions among the three agents used. As will be described later in this communication, the author carried out two experiments whereby to examine the results of applying the three agents in various combinations. No definite conclusion, however, could be drawn from this study as to the interaction of these agents. However, in the course of this study sarcomas arose subcutaneously in a few rats receiving monoiodoacetic acid alone. A consideration of the carcinogenetic mechanism leading to the production of these sarcomas led the author to a postulation relative to the initial step leading to the carcinogenesis, which he thinks can be applied to the carcinogenic agents in general.

Experiments

(A) Experiment I

In the previous study (Report I) o-aminoazotoluene (OAT) was injected as an accelerator together with glucose (G) and monoiodoacetic acid (IAc) to one and the same site. This time the author injected OAT at a place distant from the rest of the agents in order to study how OAT modifies the development of the glucose-induced sarcoma. Some animals were examined for the effect of the administration of IAc alone.

Material and Methods

Animals used were hybrid strains of rat weighing from 100 to 150 gr. They were divided as follows:

	Right back	Left back
Group I:	G+IAc	OAT
Group II:	G+IAc	
Group III:	IAc	

One cc glucose (solution in water, 20 %) and 0.5 cc IAc (solution in water, 0.4 gr / dl) were injected every other day; 5 % OAT suspension in olive oil was injected in dose of 0.5 cc every other week. For 3 consecutive days preceding the injection, these specimens were sterilized in an incubator at 56°C each time for 30 minutes. Animals received injections for 3 months, and were then placed under the author's observation. They were kept on minced rice, small amount of vegetable and water.

Result (Table I)

In Group I, 7 animals survived 200 days or more, and 4 survived 300 days or more. On the 236 th day one rat developed endothelioma (Figs. 1, 2). Two rats developed fibrosarcoma on the 252 nd and 290 th day, respectively. Of the effective total of 7 rats, in short, 3 rats developed sarcomas at the site of the administration of OAT.

In Group II, 7 rats survived 300 days or more, one of the highest longevity surviving 676 days. None of the animals developed sarcoma at the site of the injection. A rat died on the 489 th day with what was histologically determined as reticulosarcoma in the interstitium of the pancreas (Fig. 5). This tumor, however, could not be immediately ascribed to the carcinogen administered, since it was impossible to eliminate its relation to parasites.

In Group III, 7 rats survived 300 days or more, and 3 rats 500 days or more. One of the rats in this group developed fibrosarcoma on the 549 th day at the site of the administration of IAc (Figs. 3, 4).

Macroscopic appearance of the local tissue exposed to the repeated injection

Table I. Experiment I

Group	No.	Glucose		Monoiodoacetic acid		o-Aminoazo-toluene		Body weight (End)	Exp. days	Histological diagnosis
		Total† g	Inject. No.	Total g	Inject No.	Total g	Inject. No.			
I	1 ♂	9.6	48	0.096	48	0.105	7	190	290	Fibro-sarcoma
	2 ♂	9.6	48	0.096	48	0.105	7	260	465	
	3 ♂	9.6	48	0.096	48	0.105	7		252	Fibro-sarcoma
	4 ♀	9.6	48	0.096	48	0.105	7	190	474	
	5 ♀	9.6	48	0.096	48	0.105	7	200	465	
	6 ♀	2.6	13	0.026	13	0.045	3	200	236	Endo-thelioma
	7 ♂	2.6	13	0.026	13	0.045	3	225	443	
II	1 ♂	9.6	48	0.096	48	0	0	200	451	
	2 ♂	9.6	48	0.096	48	0	0	130	310	
	3 ♀	9.6	48	0.096	48	0	0	230	392	
	4 ♀	9.6	48	0.096	48	0	0	220	424	
	5 ♂	9.6	48	0.096	48	0	0	200	327	
	6 ♂	9.6	48	0.096	48	0	0	230	489	Reticulo- * sarcoma
	7 ♀	9.6	48	0.096	48	0	0	160	676	
	8 ♂	2.6	13	0.026	13	0	0	260	265	
III	1 ♂	0	0	0.096	48	0	0	255	341	
	2 ♂	0	0	0.096	48	0	0	160	387	
	3 ♀	0	0	0.096	48	0	0	130	549	Fibro-sarcoma
	4 ♀	0	0	0.096	48	0	0	195	596	
	5 ♀	0	0	0.096	48	0	0	185	391	
	6 ♀	0	0	0.026	13	0	0	165	577	
	7 ♀	0	0	0.026	13	0	0	195	447	

† total amount of the carcinogen

* a sarcoma developed in the interstitium of the pancreas

was similar in this experiment to the preceding one (Report I) so far as the injection of IAc plus glucose and the injection of IAc alone were concerned. At the site of the injection of IAc alone there was no local swelling. Instead,

there was chiefly an induration. After the cessation of the injections such macroscopic change subsided gradually, and became barely visible after the end of 200 days. The area exposed to OAT made an induration from the beginning of the injection to increase its severity as the treatment proceeded. This induration persisted long even after the cessation of the injection. It was possible to identify the OAT pigmented area with induration in the skin sections from the long surviving animals. At no time marked proliferation of the epidermis occurred.

When exposed to IAc, with or without the administration of glucose, the tissue showed a maked proliferation of fibroblasts around the subcutaneous area of necrosis within one or two months. After the lapse of 200 days the necrotic area was replaced completely by the growing fibroblasts. On the contrary, in the area exposed to OAT necrotic focus containing fat droplets persisted throughout the entire course of the experiment, with very scanty proliferation of the fibroblasts in the surrounding area, in spite of the frequent infiltration of lymphocytes. Such a picture gave an impression that the proliferation of fibroblasts was being inhibited.

While the sarcomas elicited from 2 rats of Group I, and one rat of Group III were typical fibrosarcomas, another rat of Group I developed a tumor seemingly resembling a fibrosarcoma, but with histological pictures compatible with an endothelioma—vascular structure with multiple slits. A rat of Group II developed a sarcoma in the interstitium of the pancreas, with histological picture of reticulosarcoma. The tumor cells contained oval nuclei in most cases, but not infrequently the kidney-shaped one. The cytoplasm extended projections and was spindle or stellar shaped. The projection linked with one another into a complex figure of irregular reticulum. No giant cells were present.

These tumors grew fairly rapidly, and the tumor-bearing animals died within

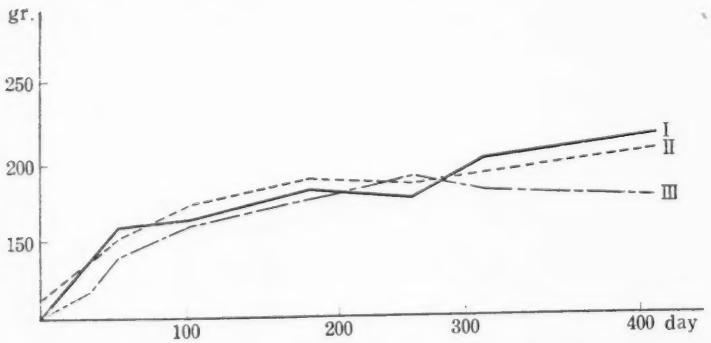

Chart 1. Average body weight of the animals (mean value) in each group (Exp. I)

half a month to a month from the time of the first occurrence of the tumor metastasis. Subcutaneous transplantation was always positive.

Chart I shows the growth of the animals in the course of this experiment.

(B) Experiment II

It was shown in Experiment I that 3 months is the sufficient length of time to evoke sarcomas by the administration of glucose plus IAc plus OAT. It was also shown that a rat developed a sarcoma after the treatment with IAc alone. Experiment II is intended to study such results in greater detail. Animals were divided into 8 groups and were injected for the period of one year.

Material and Methods

	Right back	Left back		Right back	Left back
Group I:	G+IAc	OAT	Group II:	G	OAT
Group III:	IAc	OAT	Group IV:	G+IAc	olive oil
Group V:	G+IAc		Group VI:	IAc	
Group VII:	G		Group VIII:	OAT	

Each group consisted of 13 rats weighing from 80 to 150 g. In this study the dose, interval of the injections, method of feeding were the same as in Experiment I, except that the injections were continued for 1 year.

Result (Table II)

The number of rats surviving 250 days or more were as follows: Group I 5, Group II 8, Group III 9, Group IV 2, Group V 5, Group VI 8, Group VII 8, Group VIII 11. The rate of mortality was higher in the groups receiving the combined injection of IAc and glucose than in other groups.

Two cases each of tumor occurred in Group III (receiving the administration of IAc and OAT) and in Group VI (receiving IAc alone); namely in Group III fibrosarcoma occurred on the 287th (Fig. 6) and 456th day at the site of the administration of IAc (Figs. 7, 8). In Group VI 2 rats survived 450 days or more, one of which developed a plasma cell sarcoma on the 564th day, while the other developed fibroma on the 573rd day at the site of the injection (Figs. 9, 10). Although, regretably enough, it was impossible to keep many rats alive for long period of time, owing to the high toxicity of the IAc, ability of IAc to induce cancer was fairly firmly established in this study when both of two rats surviving 450 days or longer developed the tumor. It might have been possible to induce such tumor in a greater number of rats only if they were fed properly.

On the contrary, Group I, II, IV, V, VII, VIII failed to develope a tumor even after the end of 250 days when tumor occurred usually in other experiments. While Group I appeared most apt to develop a tumor as was anticipated from the result of Experiment I, this group, strangely enough, failed to develop a

Table II. Experiment II

G.	No.	E.D.	I. No.	W.B.	W.E.	Sarcoma
I	1 ♂	489	GM 180 A 26	130	230	
	2 ♂	315	147 22	130	250	
	4 ♂	326	138 23	90	180	
	6 ♂	358	169 25	95	150	
	10 ♀	255	121 18	100	150	
II	16 ♂	387	G 180 A 26	145	200	
	17 ♂	287	138 20	120	230	
	19 ♂	583	180 26	135	275	Reticulo-sarcoma (cysticercus)
	20 ♂	387	180 26	135	140	
	22 ♂	352	169 25	105	115	
	23 ♂	351	169 25	105	130	
	24 ♀	284	138 20	105	155	
	26 ♀	394	164 26	105	110	
III	30 ♂	341	M 165 A 24	105	185	
	31 ♂	486	181 26	125	200	
	32 ♂	456	181 26	125	170	Fibro-sarcoma
	33 ♂	287	138 20	95	130	Fibro-sarcoma
	34 ♂	396	181 26	120	120	
	35 ♂	334	158 24	125	115	
	37 ♂	345	154 23	115	105	
	38 ♂	378	170 26	100	150	

G.	No.	E.D.	I. No.	W.B.	W.E.	Sarcoma
	39 ♀	325	145 23	105	100	
III	46 ♂	394	GM 175 0 26	120	200	
	50 ♀	271	122 19	115	140	
	53 ♀	324	GM 147	100	125	
	54 ♀	324	157	100	120	
V	57 ♀	329	147	90	120	
	58 ♀	411	167	105	130	
	61 ♂	286	135	90	160	
	68 ♀	345	M 166	85	120	
	69 ♀	564	180	100	185	Plasma cell sarcoma
	71 ♀	345	166	90	140	
VI	72 ♂	416	180	140	220	
	73 ♂	421	180	95	220	
	75 ♂	366	176	125	150	
	76 ♂	401	180	100	185	
	77 ♂	573	180	90	200	Fibroma
	80 ♀	389	G 181	80	140	
	82 ♂	306	133	130	140	
VII	83 ♂	359	174	70	160	
	84 ♂	396	181	90	150	
	85 ♂	401	181	80	220	

G.	No.	E.D.	I. No.	W.B.	W.E.	Sarcoma	G.	No.	E.D.	I. No.	W.B.	W.E.	Sarcoma
	102♂	331	161	130	225			91♀	464	21	120	165	
	103♂	331	161	115	195			92♀	600	26	70	160	
	104♂	324	155	125	140			93♂	278	19	125	145	
	86♀	346	A 24	90	130		Ⅶ	95♂	358	25	90	160	
	87♂	411	26	95	200			96♂	385	26	125	150	
	88♀	345	24	85	160			97♂	278	20	150	245	
	90♀	258	18	100	150			98♂	494	26	115	200	

G. group E.D. experimental days I. No. numbers of injection
W.B. body weight (the beginnig) W.E. body weight (the end)
G glucose M monoiodoacetic acid A o-aminoazotoluene O olive oil

tumor even in the long surviving animals. Since fibroblasts proliferated far more vigorously at the site of the administration of glucose plus IAc in comparison with the site of OAT administration, the former site (G plus IAc) appears more prone to sprout a tumor, even if it were the case that OAT administered was not so much effective as in Experiment I. However, if it was the case that the optimal time for carcinogenesis had passed far before the end of the injection, the local necrosis induced by the injections of IAc may have destroyed the then sprouting seed of a cancer. From these considerations the author will refrain from giving a hasty conclusion as to this problem and leave this issue to the future investigation.

A rat of Group II developed a cysticercus sarcoma on the 583 rd day (Figs. 11—14).

Both macroscopic and histological appearance of the local tissue at different stages of the carcinogenesis were the same as in Experiment I. The fibroblasts proliferated much more vigorously at the site of the administration of IAc alone or glucose plus IAc in comparison with the site of the OAT administration. No essential difference existed, however, between the former two areas. The changes at the site of glucose administration was so mild that there was no more than a mild induration after the long-continued injections of glucose. Proliferation of the subcutaneous connective tissue was also milder at this area as compared with the site of OAT administration. Effect of injecting other agents at a distant place was obscure histologically throughout the whole groups.

Of the fibrosarcoma elicited from Group III, one case was macroscopically inadequate to be called "a malignant tumor", but showed a histological picture compatible with a fibrosarcoma, with subcutaneous infiltration into the muscular layers. The cysticercus sarcoma (reticulosarcoma) occurring in Group II arose in the peritoneal cavity just beneath the liver as a ball-shaped tumor with a

diameter of 3 cm. This tumor intricated the pancreas and the transverse colon, and was linked to a lower portion on the liver through a funicular, fibrous band. This tumor contained a cysticercus. Its infiltration extended to the abdominal wall, anterior and posterior, the omentum and beneath the diaphragm. Metastasis was identified around the aorta, and in the mesenterial lymph glands. Histologically, cytoplasm was mostly oval-shaped and extended projections which linked with one another to form a reticulum. Nucleus was either round, oval or kidney-shaped, and varied in size. Nuclear net was coarse. Mitosis was noted abundantly.

The fibroma elicited from Group VI had the size of 3.6×2.7×1.1, with histological features of a marked proliferation of robust fibroblasts in distinct row, with scanty pictures of mitosis.

The plasma cell sarcoma elicited from another rat of Group VI was worthy of attention, because this was the first instance of plasma cell sarcoma ever experimentally produced in the animal. Histological and cytological appearance of this tumor, together with the result of its transplantation in rats will be presented separately.

Except the fibromas, all the tumors were transplantable.

Growth curves of the animals are shown in Chart II.

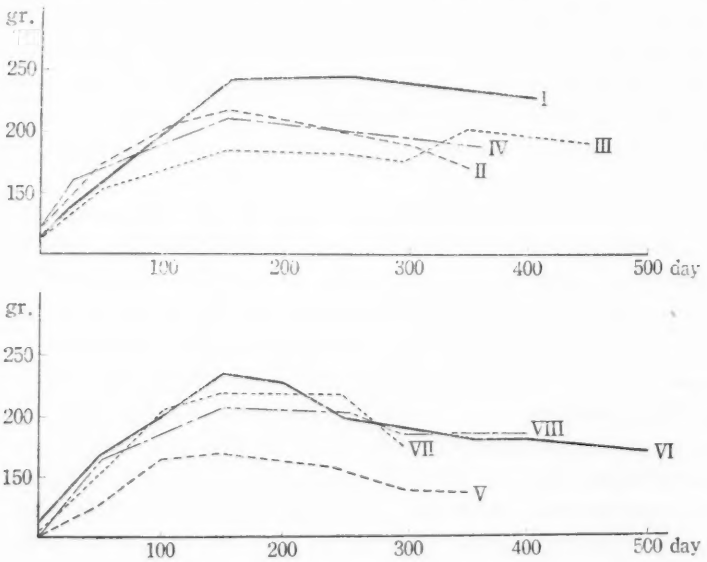

Chart II. Average body weight of the animals (mean value) in each group (Exp. I).

Summary and Discussion

In his study of the experimental production of the cancer, employing OAT

alone by the subcutaneous route, Yoshida (1934) observed that, though many animals died and a few survived long, in those surviving cancer arose with a high frequency from the skin distant from the site of the injections. In Experiment I of the author's, tumor developed in 3 of the 7 rats receiving the injection of glucose, IAc and OAT. Interrestingly enough, these induced tumors were sarcomas arising from the site of the injection. On the other hand, no tumor was elicited from the group of rats receiving OAT alone (Experiment II). Considering the results of Experiment I it appears that glucose and IAc administered in combination induce in the animal the general condition, while the OAT induces the local condition necessary for the carcinogenesis. According to the author's method, three months was the minimum period of injection-treatment necessary for a successful carcinogenesis. Results seem to be somewhat inconsistent firstly in that fibroblasts proliferated far more vigorously at the site of the injection of glucose plus IAc than at the site of OAT administration either in the 1st or the 2nd Experiment, and secondly in that the same treatment as in the Group I of Experiment 1 for one year in Experiment II unexpectedly failed to evoke a cancer. These facts may be explained in many ways, but the author feels that this problem calls for a future study in its particular relation to the interaction of the agents used.

Carcinogenicity of IAc has not yet been reported on. From the results of Experiments I and II where injection of IAc alone was carried out, it became highly possible that this agent has a carcinogenic activity. Namely, tumor occurred in one of the 7 rats receiving injections for 3 months (Exp. I), and in 2 of the 13 rats receiving the injections for one year. These tumors invariably occurred in rats surviving 500 days or more. Frequency of the occurrence of tumor, expressed in ratio to the number of rats surviving more than 500 days, is 1 out of 3 (Exp. I) and 2 out of 2 (Exp, II). It is dangerous to discuss the frequency of the carcinogenesis on the basis of these ratios, because the number of animals treated was but small. However, in view of the fact that the local tissue proliferated at the site of the administration, that tumors sprouted in good unison and that the tumor arose from the site of the injection, it may be safely concluded that IAc was the causative agent for the produced tumors. Tumor occurred in 2 of the 9 rats receiving IAc plus OAT for one year, on the 300th day and 450th day, respectively. The former tumor was barely histologically identified as sarcoma. Two rats, including the latter tumor bearing rat, survived over 450 days. However, there is no reason to conclude that the administration of OAT inhibited the otherwise emerging IAc induced sarcoma, comparing with Exp. I. It should be interpreted as an expression of the carcinogenicity of IAc.

It is a well established fact that IAc inhibits the activity of phosphoglyceraldehyde dehydrogenase in vivo as well as in vitro, and thus interrupts the glycolysis, with the resultant accumulation of hexose-diphosphoric acid. Rapkine (1933, 38) demonstrated that SH groups exist in the active group of the apoferment of phosphoglyceraldehyde dehydrogenase, and that the inactivation of this enzyme by IAc rests with the latter's assault on this SH group. Later, Barron (1951) pointed out that IAc was an important SH inhibitor. There is no definite evidence supporting the postulation that the carcinogenic ability of IAc rests with its inhibitory action on the SH group of phosphoglyceraldehyde dehydrogenase. However, the author is interested in Barron's suggestion that carcinogenetic mechanism is initiated by a ferment, such as phosphoglyceraldehyde dehydrogenase, which possesses SH groups very sensitive to SH-inhibitor. On the other hand, it can be the case that IAc combines, probably with less affinity, with SH enzyme or protein other than phosphoglyceraldehyde dehydrogenase, with the resultant deletion of such enzyme or protein, which, in turn, leads to carcinogenesis. It may be also postulated that such conjugation leads to the structural alteration of the enzyme or protein, which, in turn, leads to an altered protein-synthesis and finally to the production of a cancer.

That the SH groups of the living body substance plays a role in carcinogenesis has long been the subject of issue. Ever since the relation between SH groups and the cell proliferation became clear, importance of this problem quickly impressed many scholars. As to its relation to the carcinogenic polycyclic hydrocarbon, many indirect evidences accumulated.

That there is a close relation between the metabolism of the carcinogenic agents and the occurrence of the cancer, is a well known fact. Calcutt (1949, 50) made an interesting observation that SH group of thiol-compound was oxidized by benzpyrene, which, in turn, was oxidized and converted to a compound akin to 8; 9-dioxy-8; 9-dihydro-3; 4-benzpyrene. Recent papers (Wood, 1953; Di Paolo, 1954) reported that blood and skin level of SH group decreased following the application of carcinogenic hydrocarbons. As to the conjugation of carcinogen with the animal protein, Miller (1950, 51, 52) and Heidelberger (1953) threw a light on this problem by isolating the benzpyrene conjugate of the tissue protein. Wood and Fieser (1940), who studied the molecular structure of such conjugate, suggested that polycyclic hydrocarbon combines with RSH of animal protein at the meso-position. Following the introduction of quantum mechanical basis to the study of carcinogenicity by Pullman (1946), Boyland (1949) postulated that SH group of animal protein combines with 9; 10-dimethyl-1; 2-benzanthracene during the latter's detoxication in the living body at the highly active K region simultaneous with the occurrence of perhydroxylation at 3′; 4′ positions. This postulation is in agreement with the authors' conclu-

sion from their newly developed method of calculation of electron density (1954). The author postulates that such conjugation takes place at K region rather than the meso-position, and that conjugation with tissue protein occurs at the site of the latter's SH group. Another light was thrown on the relation between the SH group and carcinogenesis by the findings that carcinogenic activity of hydrocarbon was retarded by BAL—SH compound (Lusky, 1947), maleic acid (Crabtree, 1945), brombenzen (Crabtree, 1944) and mustard gas (Berenblum, 1929, 31)—SH inhibitor. Graffi (1939, 40) observed that benzpyrene easily combines with the cytoplasmic granules which are rich in SH protein and ribonucleic acid. In view of the fact that these granules contained respiratory enzyme with SH group, Pottor (1944) postulated that the inhibition of such enzymes leads to a carcinogenesis. In addition, SH group seems to play an important role in the carcinogenesis due to the radioactive rays, arsenics, quinone, nitrogen mustard, light green F. S., etc.

In search of a direct evidence of his postulation, the author studied the entity of so-called protein-carcinogen complex (Miller, 1947-53), by analyzing the conjugate protein secured from experimental animals fed with butter yellow by means of alkaline hodrolysis. It was thus possible to identify the presence of sulphur in the so-called polar dye. That this carcinogenic dye is conjugated with protein through the latter's S-group remains to be verified. However, it is suggested that S-compound of the living body may be involved in this conjugation, in view of the fact that sulphur was elicited from a hydrolysate of very low molecular weight.

In summary, results of the author's study employing IAc constitute indirect evidences of the SH-conjugation hypothesis of the carcinogenesis.

Conclusion

In the previous work it was suggested that the administration of monoiodoacetic acid (IAc) accelerates the development of the glucose-induced sarcoma. In the present study the author tried to elucidate the interaction among glucose (G), monoiodoacetic acid (IAc) and the o-aminoazotoluene (OAT), the accelerator. At the same time carcinogenicity of IAc as administered alone was studied. The results were as follows:

(1) The subcutaneous injections of glucose plus IAc with simultaneous injections of OAT at a distant place were continued for 3 months in rats. Sarcoma occurred in 3 of the 7 rats at the site of the OAT administration. Tumors did not occur in the rats receiving G plus IAc. From this result it is concluded that the administration for 3 months of G plus IAc induced the general condition, while that of OAT induced the local condition necessary for the development of a tumor.

(2) G, IAc and OAT were administered in various combinations to different groups of rats for the period of 1 year. Except the IAc plus OAT-group and IAc group, the majority of the groups failed to develop tumors. As to the interaction of these agents used during a prolonged course of the treatment, much remains to be studied in the future.

(3) Effect of the administration of IAc alone was studied in both of the two experiments. After the injections for 3 months tumor developed in 2 of the 3 rats surviving more than 500 days. After the injections for 1 years tumors occurred in both of the 2 cases surviving the same span of time. As to a group treated similarly for 1 year with additional OAT, sarcoma occurred in 2 of the 9 cases at the site of the IAc administration. These results constitute evidence that IAc does have a carcinogenic ability, even though it evokes tumors very slowly.

(4) As to the carcinogenetic mechanism of IAc, we have previously postulated that IAc inhibits the phosphoglyceraldehyde dehydrogenase (SH enzyme), with resulting accumulation of hexose. However, it appears equally possible to postulate that the administered IAc is conjugated with the SH group of the common proteins, with resultant deletion and / or structural alteration of the protein (enzyme) leading to the carcinogenesis. Any way, the formation of the conjugate protein (through the conjugation of a carcinogen with the SH group of the animal protein) is probably the initiating step of the carcinogenetic mechanism. This postulation may equally hold for other carcinagens in general. In order to verify this postulation, the author is engaged in the analysis of the conjugate protein secured from the butter yellow-fed animals, whereby to demonstrate directly that the carcinogen is linked to the SH group of the animal protein.

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Legend for Plates XVIII and XIX

Fig. 1: Endothelioma (Exp. I, Group I, No. 6), arising from the site of the administration of OAT on the 236th day.

Fig. 2: Histological picture of the above tumor.

Fig. 3: Fibrosarcoma (Exp. I, Group III, No. 3) arising from the site of the administration of IAc on the 549th day.

Fig. 4: Histological picture of the above tumor.

Fig. 5: Reticulosarcoma (Exp. I, Group II, No. 6), developing in the interstitium of the pancreas on the 489th day.

Fig. 6: Fibrosarcoma (Exp. II, Group III, No. 33), arising from the site of the administration of IAc on the 287th day.

Fig. 7: Fibrosarcoma (Exp. II, Group III, No. 32), arising from the site of the administration of IAc on tne 456th day.

Fig. 8: Histological picture of the above tumor.

Fig. 9: Fibroma (Exp. II, Group VI, No. 77), arising from the site of the administration of IAc on the 573th day.

Fig. 10: Histological picture of the above tumor.

Fig. 11: Cysticercus sarcoma (Exp. II, Group II, No. 19), 583 days.

Fig. 12: Histological picture of the above tumor; reticulosarcoma.

Fig. 13: Giemsa-stained smear of the above tumor cells.

Fig. 14: Phase-contrast-microscopic observation of the above tumor cells.

要　旨

発癌組織における糖代謝酵素系の切断に関する実験的研究（第 II 報）
附：発癌過程における SH 基結合説について

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1938 年，西山氏が濃厚葡萄糖皮下注射による肉腫発生に成功して以来，糖原，乳糖，果糖の如き生理的に重要な糖類についてもその発癌性が相次いで知られるようになった。しかしその発癌の機序に関しては今日ほとんど追究がなされていないといって過言ではない。そこでわれわれは，葡萄糖が生体内で分解をうけるに当り，如何なる段階のものが発癌に関与しているかを明かにするために，糖代謝酵素系の中断を行うと考えられるフツ化ソーダまたは沃度醋酸を葡萄糖と同時に負荷することによって，およそ葡萄糖の三炭糖に分解される以前の段階が発癌に与っていることを明かにした（第 I 報）。しかしこの実験においては，o-アミノアゾトルオールを発癌促進剤として葡萄糖，沃度醋酸と同じ注射部位に適用したため，この三者が発癌に際して，相互に如何に作用し合っているかをさらに検索することが必要となった。そこで下記の如く，前後二回にわたって発癌実験を試み，若干の結果を得たが，それらの相互作用については確定的な結論を見出すには至らなかった。しかし他方，これ等の実験において沃度醋酸単独でラッテの皮下に肉腫を発生せしめ得ることが明かとなり，その発癌機構の考察により，広く一般の発癌性物質にまで及ぼし得る発癌の生化学的過程の第一段階を新たに想定することができた。

第 I 実験	右背皮下	左背皮下
I 群	葡萄糖＋沃度醋酸	o-アミノアゾトルオール
II 群	葡萄糖＋沃度醋酸	
III 群	沃度醋酸	各群 8 例

葡萄糖は 20 % 水溶液とし沃度醋酸は 0.4 gr./dl. 水溶液としてそれぞれ 1 cc，0.5 cc 隔日に注射，OAT は 5 % オリーブ油溶液として 0.5 cc 隔週に注射した。注射期間は 3 カ月，その後は単に飼育するにとどめた。

第 I 群では 200 日以上生存せるものは 7 例で，その中 3 例に実験日数 230～290 日で OAT 注射部位に一致して肉腫（血管内皮腫 1 例，繊維肉腫 2 例）を発生せしめ得た。第 II

群では 300 日以上生存せるものは 7 例で，最高 676 日まで生存せしめ得たが注射部位に肉腫の発生せるものは 1 例もなかった。489 日で斃死した 1 例は膵間質に細網肉腫が組織学的に証明せられたが発癌物質の作用によるものとは決定し難く，寄生虫との関係も否定し得なかった。第 III 群では 500 日以上生存せる 3 例の中 1 例に沃度醋酸注射部位に一致して繊維肉腫を生じた (実験日数 549 日)。以上の結果から，かかる条件においては葡萄糖，沃度醋酸の併用注射は，動物に発癌に必要な全身的要約を与え，OAT は局所的要約を与えると考えられ，また 3 カ月という短い注射期間でも発癌には充分有効であると考えられた。さらに 1 例ではあるが沃度醋酸単独で注射部位に肉腫を発生せしめ得たことは，該薬物の発癌性を予想せしめるものとして興味深い。

第 II 実験

第 I 実験では注射期間は僅かに 3 カ月であったが，本実験においては注射期間を 1 カ年とし，かつ多数の組合せの下に発癌実験を行った。

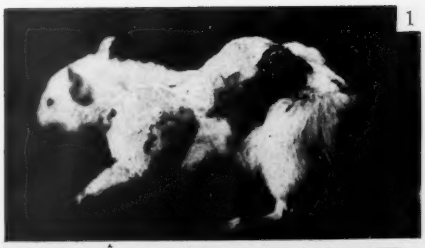
	右背皮下	左背皮下
I 群	葡萄糖＋沃度醋酸	o-アミノアゾトルオール
II 群	葡萄糖	o-アミノアゾトルオール
III 群	沃度醋酸	o-アミノアゾトルオール
IV 群	葡萄糖＋沃度醋酸	オリーブ油
V 群	葡萄糖＋沃度醋酸	
VI 群	沃度醋酸	
VII 群	葡萄糖	
VIII 群	o-アミノアゾトルオール	各群 13 例

注射期間が 1 カ年である以外，注射量，注射間隔，飼育法は第 I 実験と全く同様であった。

本実験においては第 I 実験に比し動物の斃死率は一般に大であったが，沃度醋酸，OAT 併用注射群である第 III 群，及び沃度醋酸単独注射を行った第 IV 群で各々 2 例沃度醋酸注射部位に一致して腫瘍の発生をみたことは注目に値する。すなわち，第 III 群では 250 日以上生存せる 9 例中 2 例に，それぞれ 287, 456 実験日に繊維肉腫を生じ，第 VI 群では 500 日以上生存せる 2 例の中 1 例に形質細胞肉腫を，1 例に繊維腫を発生した。これに反し他の群では，第 I 実験の成績から按じて発癌期と考えられる 250 日以後においても，第 II 群に 585 実験日に発生したチスチセルクス肉腫を除き，1 例の腫瘍発生もみなかったことはやや理解に困難な現象である。その原因として幾つかの理由を挙げ得るが，発癌性物質の相互作用についてはなお将来の研究が必要であろう。

沃度醋酸の発癌性について今日まで報告せられたものは皆無である。われわれの実験結果から直ちに発癌率を云々することはもとより不可能であるが，注射局所の増殖性変化，ほぼ一定の発癌潜伏期，及び注射局所での発癌という事実より按じて沃度醋酸による肉腫発生と断じて誤りはないであろう。かつまた適当な飼育を行えば高率の発癌を期待することも不可能ではない。さてその発癌機序について考えてみるに，従来のわれわれの構想よりすれば，燐グリセルアルデヒド脱水素酵素（SH 酵素）の抑制による六炭糖の蓄積によると考えられるが，他方沃度醋酸はひろく蛋白 SH 基との結合が考えられるので，その結合による（燐グリセルアルデヒド脱水酵素をも含めて）酵素の抹殺乃至は蛋白構造の変化が発癌へ導くとの推定も可能である。

発癌過程の初期の段階に生体の SH 基が重要な役割を果しているということは，今日まで他の多数の発癌剤について述べられている所である。この意味で SH 基抑制剤として古くから知られている沃度醋酸によって発癌せしめ得たというわれわれの結果は，発癌性物質の SH 基結合説に対して有力な間接的根拠を与えるものといいうるであろう。（文部省科学研究費による）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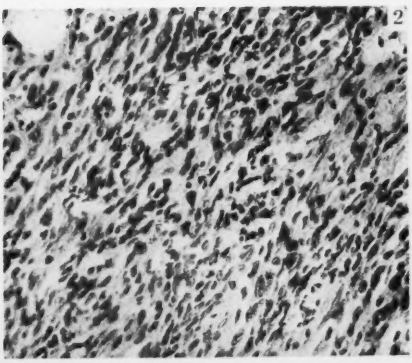
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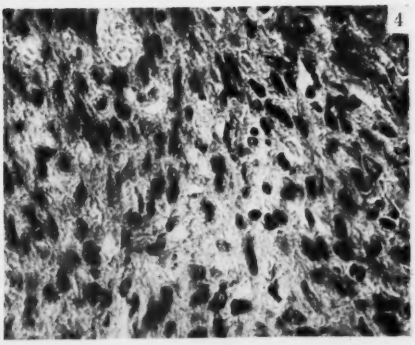
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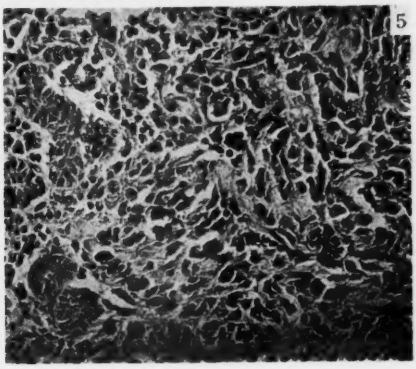
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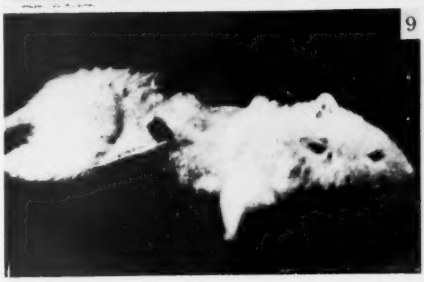
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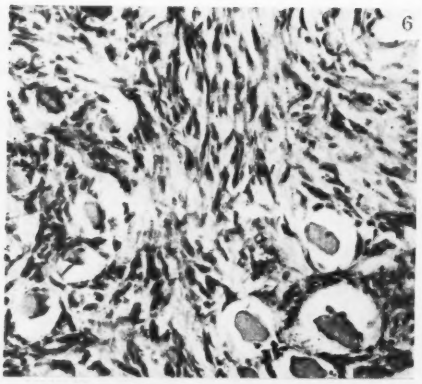
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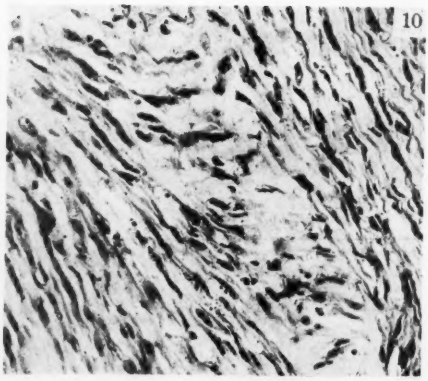
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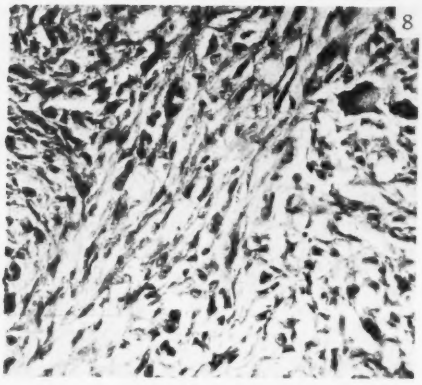
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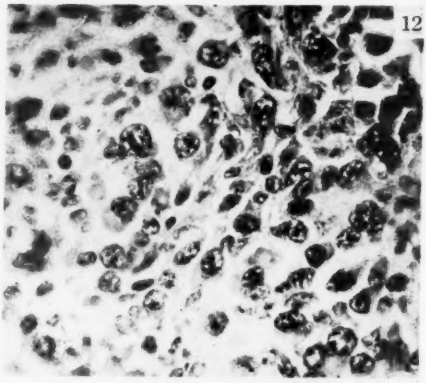
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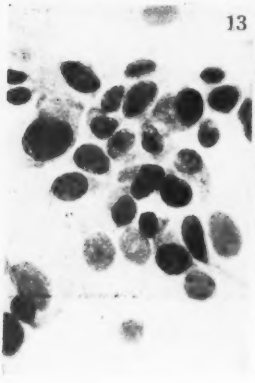
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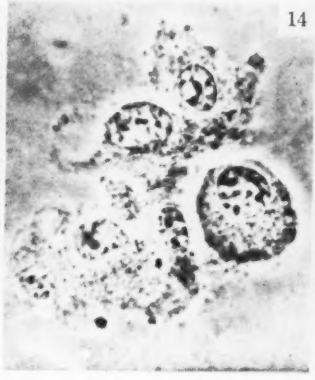
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[GANN, Vol. 45; December, 1954]

A CASE OF TRANSPLANTABLE PLASMA CELL SARCOMA PRODUCED EXPERIMENTALLY IN A RAT
(With Plates XX and XXI)

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Introduction

With exception of a report presented by Bloom (1947) that a plasma cell tumor occurred in the humerus of a male dog pertaining to English setter, no investigator ever reported on the incidence of this kind of tumor in animal (Engelbreth-Holm, 1942). During a serial study on the glucose-induced sarcoma of rat with primary stress on the role of monoiodoacetic acid in the sarcogenetic mechanism, the author encountered a tumor of the histologic picture of plasma cell sarcoma arising in a rat subjected to repeated injections of monoiodoacetic acid alone. The author successfully transplanted this tumor serially in rats until it died out after 7 generations. That this tumor arose primarily in the subcutaneous tissue and that fairly extensive study was made on the cytological features of this tumor, induced the author to present this paper.

Experimental Method and Material

This sarcoma arose during the course of the study described in "IInd Report" (preceding communication, Tagashira, 1954). The carcinogens employed in this study were: concentrated glucose solution injected subcutaneously for the purpose of evokig a glucose-induced sarcoma (G); o-aminoazotoluene injected subcutaneously in order to facilitate the sarcogenesis (A); and monoiodoacetic acid, newly established carcinogen by the author (1954), injected subcutaneously in order to inhibit enzyme systems involved in the metabolism of glucose (M). Animals were divided into 8 groups each receiving different combination of carcinogens. Namely, Group 1 received all of the carcinogens (G+M+A); Group 2 (G+M); Group 3 (M+A): Group 4 (G+M+olive oil); Group 5 (G+M); Group 6 (M); Group 7 (G); Group 8 (A). Frequency of the incidence of the induced tumor in each group was discussed elsewhere. Most tumors evoked by the above listed carcinogens, according to the authors experience, are either one of fibroma, fibrosarcoma and polymorphocellular sarcoma. It happened that a plasma cell sarcoma occured in a rat from Group 6 (No. 69) receiving monoiodoacetic acid alone subcutaneously in dose of 0.5 cc (0.4 g/dl) every other day for the period of one year (180 injections).

Following the cessation of the injections, this group had been placed under the author's observation, and it was around the 540 th experimental day that a palpable nodule arose in one of the rats at its right back exactly at the site of the subcutaneous administration of monoiodoacetic acid. Thenceforth this tumor increased its size progressively and reached the size of 3.2×2.4×1.1 on the 556 th day when it was felt elastic hard in the absence of conspicuous vascular dilatation on its surface. This tumor mass softened for most part around the 562 nd day, and the animal died on the 564 th day when it weighed 185 g, while its pre-treatment weight was 100 g. Sections were prepared from the tumor and studied histologically. Pieces of the tumor were transplanted in 3 rats either by subcutaneous or by intraperitoneal route.

Findings at the Necropsy (Text-fig. 1)

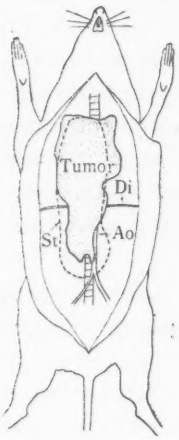
The primary tumor arose in the subcutaneous tissue and had the size of 3.6 ×3×1.6 cm without any conspicuous vascular dilatation. There was a focus of viscious, bloody fluid together with that of soft, necrotic mass each occupying about one-third portion of the tumor. Tumor was adherent to the underlying tissue, and was encapsulated by a thick layer of the connective tissue. The skin covering the tumor could be peeled off easily. The tumor was found growing through the muscular layer into the abdominal cavity. It invaded the retroperitoneal cavity and grew into the right posterior abdominal wall to extend further along the abdominal aorta and into the entire area of the diaphragm to form a massive tumor in the anterior mediastinum wherein to implicate the heart and medial portions of the lungs.

Left lung was invaded by the tumor by way of the diaphragm and the hilus. Right lung was invaded in a simlar way, but somewhat weakly. Distinction was sharp between the tumor invading the lungs and healthy portion of pulmonary parenchyma. Nodular metastases spread in the parenchyma of the lungs. The tumor mass in the pleural and abdominal cavities appeared whitish gray and was felt elastic soft and there was scanty signs of necrosis. Pleural effusion and ascites were absent. One of the peri-aortic lymph nodes was tumorous with histologic evidence of metastasis. In the absence of enlarged axillary or inguinal glands, metastasis was established histologically in the axillary glands. Vertebrae were intact.

The liver weighed 8.7 g, with histologic signs of congestion associated with hypertrophy of the stellate cells. The spleen weighed 1 g and was characterized by congestion and atrophied follicles. Kidneys weighd 1.4 g (right), and 1.2 g (ieft). Renal capsule was adherent to the tumor, but parenchymatous infiltration was absent. Omentum and mesenterium were intact.

Microscopically, bone marrow (vertebrae, femur) was intact in its cellular constitution. The kidneys did not contain such protein crystals and giant cells as

Text-fig. 1
Invasion of the tumor

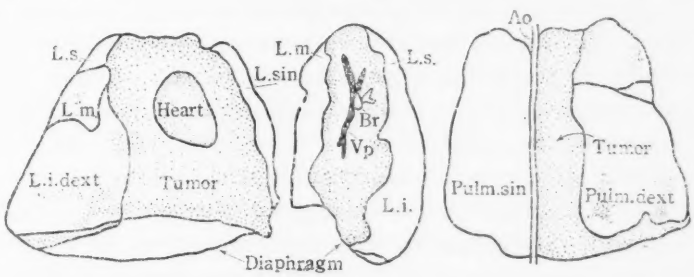


are elicited from the kidneys of human subject bearing plasma cell myeloma. There were occasional renal tubules containing hyaline cylinders (Fig. 4).

Histological and Cytological Findings of the Tumor

Data of the supravital observation are not available as to the tumor earlier than the 5th transplant generation. Since there is no essential difference in the histological appearance of the tumor cells from a generation to another so far as smear and sections are concerned, histological findings of this tumor are summarized in the following paragraphs.

The induced tumor grew into the subcutaneous muscle layers on one hand (Fig. 2), and extended through the retroperitoneum and into the mediastinum per continuitatem on the other. The appearance of this growth bears a perfect resemblance to a sarcoma. Owing to the fact that repeated injections of monoiodoacetic acid resulted in the necrosis associated with induration of the local tissue, it could hardly be determined whether the primary location of the scar tissue was intramuscular or exactly subcutaneous. In view of the fact that tumor carried a capsule superficial to it, however, it appears rather likely that this tumor arose from a scar tissue deep in the muscle layer, and grew deeper into the body cavity. The area invaded by the growth was extensive, but the pathway of the infiltration is characteristically interstitial, while parenchymatous organs remain fairly intact. Massive tumors in the retroperitoneum or the mediastinum did not grow into the kidneys or the heart beyond their capsules. Either lung escaped a deep infiltration save its hilar region. Tumor invaded no lymph glands other than those draining the area involved in the primary tumor. Generally it appeared that the growth was vigorous in the interstitial tissue, but slow in the parenchymatous organs.



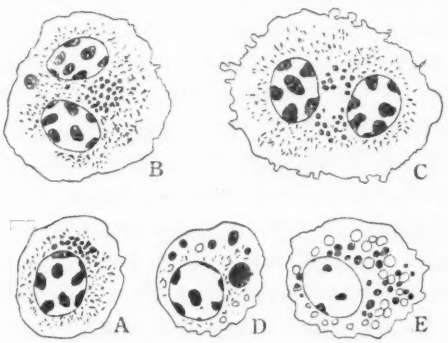
Histologically, rich network of silver fiber was present in tumor tissue, probably because the tumor grew into the interstitial tissue which is abundant in

connective tissue. However, exact relationship between tumor cells and silver fiber was indistinct (Fig. 3). Appearance of the growth through the muscle layers of the body trunk resembled human chloroma.

Cytologically, tumor cells presented a picture compatible with a plasma cell with characteristically scanty cytoplasm as is usually elicited from plasma cells migrating in the inflammatory focus. There is a clear zone adjacent to the nucleus which is invariably cart-wheel like with nucleoli somewhat smaller than those elicited from myeloma cells in man. There are occasional cells simulating giant cells with relatively narrow cytoplasm. Inclusion body was absent (Fig. 1).

Giemsa-stained smear presented microscopically identical pictures with sections (Fig. 7). The cells contained somewhat small, basophilic cytoplasm, and cart-wheel like nuclei. Nucleoli were hardly detectable. Perinuclear clear zone was generally small. Inclusion bodies were absent. Tumor cells cotained cart-wheel like nuclei, but none of them exhibited fine nuclear net similar to our "lymphogonia." Dividing cells were encountered abundantly in sections secured from vigorous-tumor-bearing animals, while scanty cell division occurred in sections secured from regressing-tumor-bearing animals. Tumor cells took methyl-green pyronine stains in a way compatible with normal plasma cells. Peroxidase reaction was negative.

Supravital observation revealed the following pictures (Text-fig. 2): mitochondria stained by janus-green were distributed fairly extensively throughout the cytoplasm, in a lower frequency, they clustered around the nucleus. A minority of neutral-red vacuoles occured in the perinuclear clear zone either sparsely or in groups. Carbon particle phagocytic test (employing film-method) was negative.



A; B, C——Tumor cells; two of them binucleated. D, E——Degenerated tumor cells. Mitochondria: rods, Neutral red vacuoles: black and rounded, vacuole degeneration: rings.

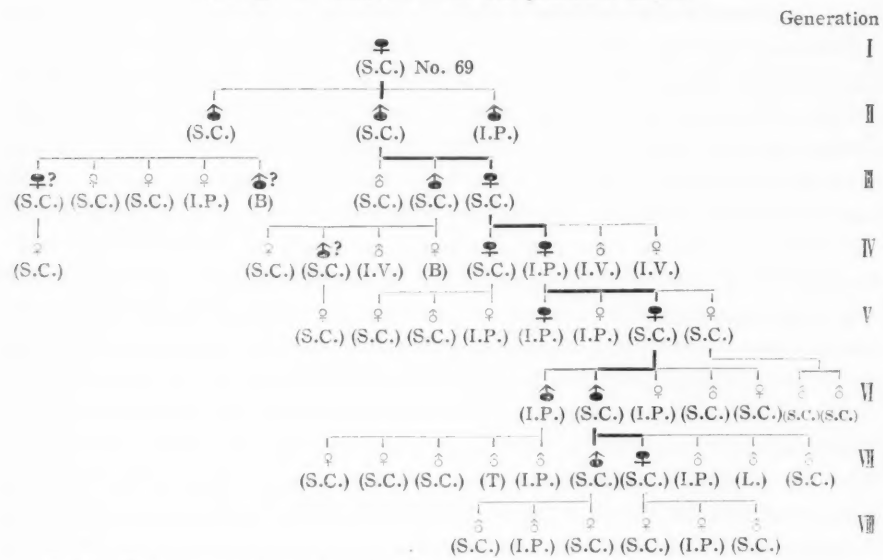
Phase contrast microscopy made a detailed observation of mitochondria possible (Fig. 8-13). The predominating numbers of mitochondria were rod-shaped, somewhat smaller and narrower in shape than those contained in normal plasma cells. It is reported that, at the time of mitosis, mitochondria increase in number and become somewhat slender in shape (Dalton, 1951). This kind of change was noted in an exaggerated degree in this tumor cells. The author studied the morphologies of cells at different stage of mitotic division. As was reported by Amano, Unno, Hanaoka previously (1951), the nucleus of plasma cell normally contains chromatine mass which hangs from its nuclear membrane like stalactite, and this obscures the morphologies of the nucleus at prophase. It was difficult, therefore to observe the distinct morphologies of chromosomes at the stages equivalent to the 1st and 2nd stage of usual tumor cells under mitotic process. The 3rd stage of contraction of the chromosomes immediately before metaphase was the first sign of the prophase noted by the author. This fact may be partly attributable to the fact that the nucleus of a plasma cell is physiologically in a state of fused prochromosomes (Amano, 1954) and it completes the prophasic process very rapidly. On the other hand, phase contrast microscope elicited detailed figures of the cell division at telophase where the loosening of chromosomes occures. Supravital observation depicted occasional tumor-cell-nuclei taking neutral-red stains at an early stainning.....suggestive of degeneration..... Such nuclei, phase-contrast-microscopically, lost chromatin mass rapidly and turned into transparent bodies containing a small nucleolus. This probably means a disentangle of chromatine blocks through the only loss of tonicity of the chromosomal structure. The like of such pictures had not been encountered in the mitotic division of plasma cells appearing in the infiammatory focus. These facts depict an aspect of the degeneration of the neoplastic plasma cells and presents an interesting problem with regard to the cytological natures of the plasmacytic nucleus.

Transplantation ot the Tumor

Since pieces of the primary tumor were transplanted from rat No. 69 after its death, resulting tumor was superimposed by mixed infection. Probably because of this infection, tumor regressed in the 2nd generation. Removal of the infection was attempted by the administration of penicillin, streptomycin in the 3rd generation, with the result that tumor regained its vigor in the 4th generation. Intravenous transplantation of the tumor from regressing-tumor-bearing animals in this period faild to take in hosts. On the other hand, pieces of the tumor transplanted intraperitoneally from vigorous-tumor-bearing animal of the 4th generation produced a marked anemia in hosts on the 15th post-transplantation day associated with extramedullary hematopoiesis in the spleen

(Fig. 5). Bone marrow (Fig. 6) and other hematopoietic organs were free from

Text-fig. 3: Chart of serial transplantation in rats



S.C.....Subcutaneous transplantation
I.V.....Intravenous transplantation
I.P.....Intraperitoneal transplantation
L. .....Transplantation into liver
T. .....Transplantation into testis
B. .....Transplantation into bone marrow

neoplastic invasion. This fact is interesting as compared to the fact that plasma cell myeloma of man induces a marked anemia of the patient.

The results of serial transplantation are given in Text-fig. 3. Analysis of this table suggests that successful takes occurred only in transplantation by subcutaneous and intraperitoneal route, whereas the liver, testis and blood rejected a take. It may be of interest to discuss briefly the amount of tumor tissue to be implanted to secure a successful take. Namely, pieces of tumor implanted in an amount smaller than several trocars failed to take in hosts. This fact suggests that a successful take requires a fairly large amount of the tumor tissue. The rats used in this study, including both 1st and the subsequent serial generations, were all hybrids. The author feels that neoplasm derived from a highly differentiated tissue like blood-cell should have been produced and transplanted serially in an inbred strain of rats.

Summary and Discussion

That the histological appearance of the cells predominating in this tumor

are compatible with plasma cells has ample evidences. Another characteristic feature of this tumor is its slow proliferation as was shown in the result of the transplantation experiment. In view of these two features this tumor may be concluded to be a neoplasm derived from a blood cell type with proliferation not leukemic, but simulating sarcoma of fairly mild malignancy. For these reasons the author designates this tumor as "plasma cell sarcoma" or "extramedullar plasmoma."

Supravital appearance of the mitochondria in the plasma cell myeloma of man was reported by Fukase (1949). In comparison with the reported appearance, the author's tumor appeared more sarcomatous in that it contained smaller, but more numerous mitochondria. Since Yoshida sarcoma is now accepted as a monocyte-sarcoma, so is the author's tumor compatible with a plasma cell sarcoma. It is regretted that this tumor died out after 7 generations. There must have been some means to preserve the strain of this tumor, since Furth successfully transplanted leukemia of mouse serially for many generations of inbred strain.

It deserves attention that this tumor arose primarily in the subcutaneous tissue exposed to the repeated doses of monoiodoacetic acid and in the absence of bone marrow involvement. This fact sharply contrasts to the fact that most plasma cell tumors in man are derived from the bone marrow in the form of a myeloma with the exception of a plasma cell sarcoma reported by Maresch (1909) to have invaded lymph glands systematically, and a non-myelogenic plasma cell sarcoma arising primarily in the connective tissue of the neck (Kagamiyama, unpublished data).

Since plasma cell derives its origin from the adventitia cells around the blood vessel (Amano, et al, 1944), plasma cell sarcoma arising primarily from tissues other than bone marrow is no surprising matter. It is rather strange that plasma cell sarcoma like this is considered to be so rare.

Plasma cell myeloma of man is classified into γ-globulin type and β-globulin type. The author performed assays of globulin fraction, both qualitative and quantitative, on this tumor. With a cooperation of Dr. Hanaoka the author examined blood samples obtained from tumor-bearing animals of the 2nd generation on the 15th post-plantation day for their protein fractions electrophoretically. There was no evidence of increasing globulin fraction. The author does not intend to generalize this fact on the entire strains of the induced-tumor, since the tumor-bearing-animals used in this assay had received repeated doses of antibiotics in order to subdue the superimposing mixed infection and the tumor itself was regressing at the time of this assay. After the 4th generation tumor regained its vigor, but no electrophoretical analysis of the protein fractions was made either on blood or the tumor extracts.

That hybrid strains were used in this study constituted the primary reason for the author's failure to transplant this tumor for many generations. Another experiment is being contemplated employing inbred strain of animal. During the course of this study occasional cysticercus sarcoma (reticulosarcoma) of the liver occurred. This tumor could be transplanted from many generations without any particular difficulty, contrasting sharply to the failure of the plasma cell sarcoma to be carried beyond 7 generations despite the best caution employed by the author. In this failure the highly differentiated character of the tumor cell—a blood cell type—probably plays a part. With this failure, this study still merits a publication, since occurrence of plasma cell sarcoma has not reported on.

Conclusion

(1) Monoiodoacetic acid, a carcinogen, was injected repeatedly for a long period of time to a group of rats from hybrid strain. One of the rats developed a plasma cell sarcoma at the site of the injection.

(2) This tumor was consisted of predominant cells which bear a striking cytological and histological resemblance to the tumor cells of the plasma cell myeloma of man, except that the former cells contained smaller nucleoli. This tumor neither arose from the bone marrow nor metastasized in the bone marrow, but occured as a diffse, continuous infiltration in the connective tissues.

(3) Abundance of mitochondria in the tumor cell suggested a higher malignancy of this tumor in comparison with the known plasma cell myeloma of man. Sections prepared from this tumor contained abundant cells under mitotic division. Such features of the tumor sharply responded to the vigor of the growth.

(4) Blood samples obtained from regressing-tumor-bearing animals were assayed for protein fractions. No marked change was present. It is conjectural, however, whether this is attributable to a decreased vigor of the growth or to a high degree of the anaplasia.

(5) Pieces of the tumor were transplanted subcutaneously, intraperitoneally or into the testis, liver, bone marrow or blood of hosts. Successful takes occurred following the implantation by subcutaneous and intraperitoneal routes, whereas other routes rejected a successful take. Transplantation to the 7th generation was successful only in small percentage of the animal, and the tumor died out after the 7th transplant generation.

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Legend for Plates XX and XXI

Fig. 1: Microscopic picture of tumor cells in section (hematoxyline-eosin stainning); typical cart-wheel like nuclei.

Fig. 2: Tumor-cell-infiltration into the subcutaneous muscle layer. (1 st generation).

Fig. 3: Silver stainning of the tumor tissue (Akazaki's modification).

Fig. 4: Kidney, renal tubulus containing hyaline cylinders (1 st generation).

Fig. 5: Spleen, extra-medullary hematopoiesis (4th generation, 15 days after the intraperitoneal transplantation).

Fig. 6: Bone marrow (same as above).

Fig. 7: Giemsa-stained smear of the tumor cells.

Fig. 8—Fig. 13: Observation by phase contrast microscope. Fig. 8, 9—mitotic cells, Fig. 10—binucleated cell, Fig. 13—a pair of asymmetric cell division. (4th generation)

要　旨

実験的に発生せるラッテの可移植性形質細胞肉腫

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動物の形質細胞腫は Bloom が犬の大腿骨髄に自然発生したものを報告しているにとどまり，その他に記載をみない。われわれは葡萄糖肉腫の発生過程において，一沃度醋酸が如何に作用するかという点を実験的に検索中，単に一沃度醋酸のみを注射した一疋のラッテから悪性形質細胞腫と称すべきものの発生を認めた。

この動物の実験条件をのべると，一沃度醋酸 0.4 gr/dl 0.5 cc を隔日に皮下注射 180 回 1 年に及び，後放置，観察中，実験日数 540 日頃に右背皮下の注射部位に相当して，腫瘤を触知した。その後急速に大さを増し，556 実験日には 3.2×2.4×1.1 cm となり，硬度は弾力性掌で皮膚面の著しい血管拡張は認め得なかった。562 実験日に腫瘍は一部を除き軟化し始め，564 実験日において斃死した。初期体重 100 g，末期体重 185 g である。腫瘍組織は直に組織学的検索に供するとともに，他方三疋のラッテに皮下乃至腹腔内腫瘍移植を試みた。

剖検時皮下原発腫瘍の大さは 3.6×3×1.6 cm で粘稠血性の液状物及び軟い壊死状物をそれぞれ腫瘍の 1/3 程度含有し，皮下の底部に固着している。腫瘍自体は厚い結締織性の被膜を被り皮膚との癒着はないが，底部において筋層を破って腹腔内に浸潤している。後腹膜腔内に入った腫瘍は右側後腹壁に浸潤し，さらに腹部大動脈に沿って上行，横隔膜を全面において腫瘍化せしめてこれを突破し，前縦隔洞に大腫瘍を形成し，この中に心臓を擁し，さらに両肺内側を完全に侵している。肺は横隔膜面及び肺門から実質内に腫瘍浸潤を受けているが，肺実質と鋭利に境されており，その他に実質内の転移結節の撒布もみられる。胸水，腹水はないが旁大動脈淋巴腺の腫脹せるもの１個があり，腫瘍転移を認めた。腋窩，鼠蹊淋巴腺の腫大は認めなかったが，腋窩淋巴腺には組織学的に腫瘍転移を認めた。その他の臓器，例えば心，肝，脾，腎，骨髄等にはいずれも転移形成はなかった。

腎では人の形質細胞腫例で注意されているような蛋白結晶，巨細胞などは認めないが，その細尿管には硝子円柱が散見された。

固定切片標本の所見では腫瘍細胞は炎症組織におけると同様の比較的胞体の狭い形質細胞の型をとり，核周明庭を有し，核はいずれも定型的車輻状核で，人の形質細胞腫の如き大型の仁は認め難い。この間に巨細胞化せるものが混在するが，その場合には概ね胞体が狭小となって

いる。核分裂像もしばしば認められる。細胞内封入体はない。鉱銀繊維は一見多いように見えるが，腫瘍が間質内を発育しつつある関係で残存結締織に割込むことと関係あるらしく，個々の細胞との関係は明かでない。

塗抹ギームザ染色所見も切片所見とほぼ同様であるが，ペルオキシダーゼ反応は陰性で，メチール緑，ピロニン染色の態度は正常形質細胞と異るところはない。

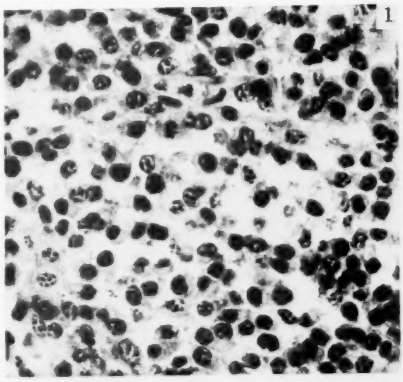
超生体染色所見では，ヤーヌス緑染色糸粒体は比較的広く胞体内に分布するが，時に核周に集るものもある。中性赤空胞はその核周明庭部に数個集合性乃至分散して認められるものがあるが，これは一部である。墨粒貪喰能は陰性である（墨膜法による）。

位相差顕微鏡によると糸粒体の状態はさらに明瞭で，正常形質細胞に比して細い短桿状の糸粒体が極めて多数認められる。元来糸粒体は分裂細胞では細短桿状化し数を増すが，この場合にはその傾向が著しい。なおこの方法で核分裂の各期を観察した。元来形質細胞の核膜には色素塊が鐘乳石の如く垂れ下っているが，この性質のために分裂前期の態度を明確に把むことは困難で，一般腫瘍細胞核に見る染色体の第一乃至第二収縮期の染色糸像を把えることは困難であった。そして第三収縮状態（中期への移行期）から初めて前期として確認し得る。これは天野が述べている如く形質細胞核が融合性前染色体状態にあり，容易に前期を通過して中期に至るためと考えられる。これに対して末期の染色体のほぐれてゆく像は確認し得た。

腫瘍細胞の移植は主として腹腔，皮下において成功し第七代まで移植可能であった。その睾丸，肝，骨髄，血液内等の移植も試みたがすべて失敗に終った。また移植動物の血液並びに腫瘍組織の蛋白像を電気泳動法により検索したが，グロブリン分屑における著明な変化を認め得なかった。唯，第4代の腹腔内移植例において移植後15日で強度の貧血を来し，脾においても髄外造血像を認めたことは人の形質細胞腫が高度の貧血を伴う事実と比較して興味深い。

以上の細胞学的性状から本腫瘍細胞が形質細胞の性状と一致することはほとんど疑を入れないところであるが，その増生の性格が比較的緩徐であることから，一血球種の腫瘍性増生ではあるが白血性ではなく，むしろ比較的悪性度の強くない肉腫，従って形質細胞肉腫と呼ぶことが出来よう。また天野の述べる如く形質細胞が血管外膜細胞から発生するものである以上，それが骨髄腫以外の形で発生することのあるのは当然で，むしろその頻度の少きに疑問のある程である。累代移植が細心の注意にかかわらず第7代までしか行われず，ために腫瘍蛋白等について詳細な研究を行い得なかったことは返す返すも残念であるが，これは本腫瘍の母細胞が血球という高度に分化した細胞であったということも大きな理由である。従ってかかる腫瘍は純系において発生せしめ，かつ同系動物に移植することが必要で，この点失敗に鑑み今後考慮すべきことであろう。

（文部省科学研究費による）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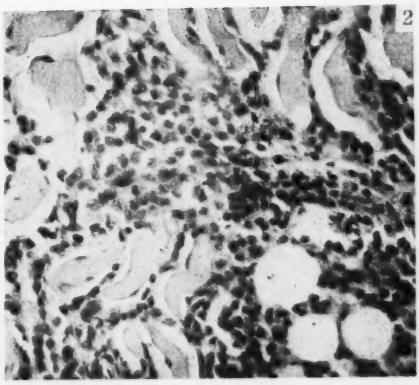
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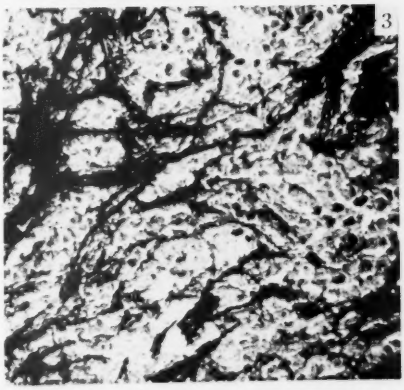
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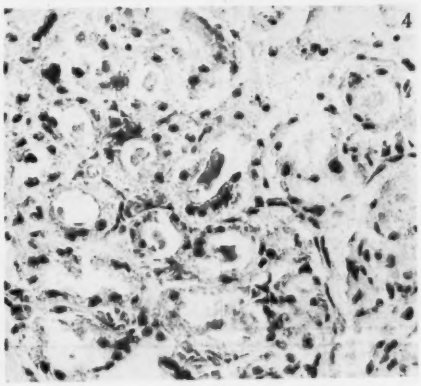
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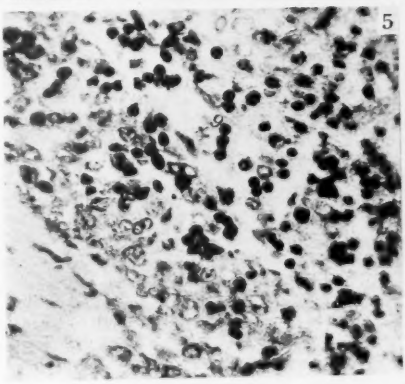
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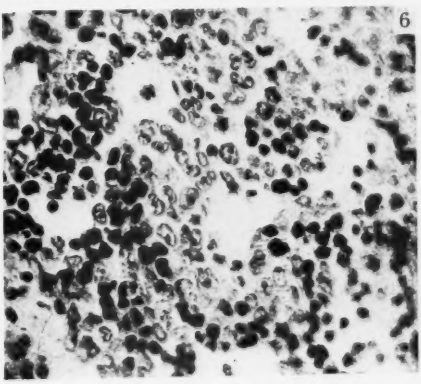
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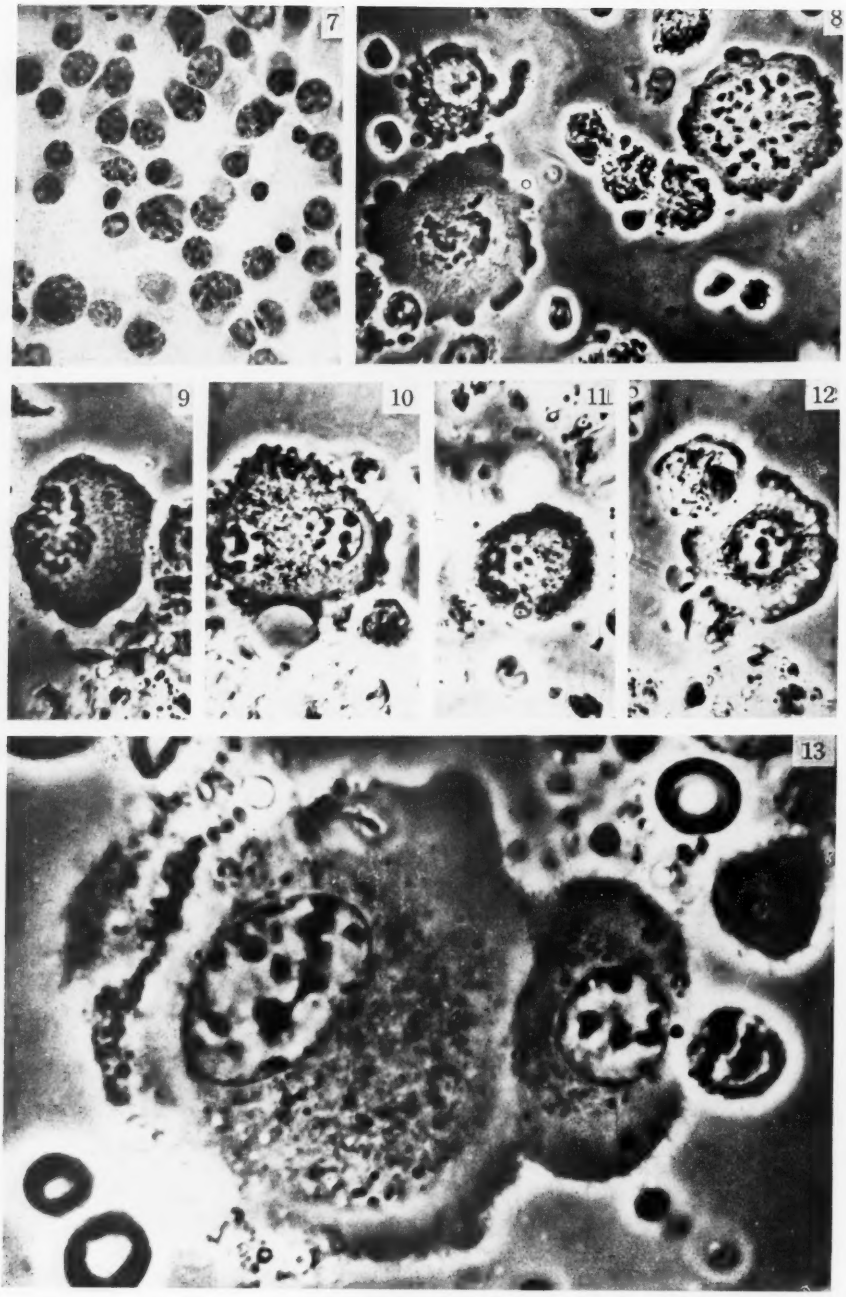
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[GANN, Vol. 45; December, 1954]

GUANASE AND ARGINASE OF LIVER OF RATS FED HEPATIC CARCINOGENS

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The present study is an attempt to determine the relative activity of guanase and arginase of liver of rats fed on 2-acetylaminofluorene (AAF) or p-dimethylaminoazobenzene (DAB) during the course of the production of liver cancer.

Material and Methods

A group of normal male albino rats was maintained on the AAF rice diet (0.3–0.5 g AAF in 20 cc of polyethylene glycol per 1 kg of polished rice) for 6 months so as to produce liver cancer. After the period of AAF feeding, the animals were fed on a normal rice diet without the carcinogen for two months. Another group of animals was kept on the diet containing DAB (0.6 g per 1 kg of rice diet) for 4 months by the routine method, and then for an additional month animals were fed on the basal diet free from the dye. In both groups water was freely and green vegetables were occasionally supplied.

Animals were exsanguinated by decapitation and then all livers were carefully examined at the time of autopsy. To show the nature of the liver findings, grossly, four grades of the pathological changes leading to the production of liver cancer were indicated by adopting the four grades of liver findings as described by Wilson and associates (1), that is: i) no hyperplasia, ii) slight or moderate nodular hyperplasis, iii) marked nodular hyperplasia and iv) liver cancer. If the liver contained more than one lesion of the four, the different portions were dissected out and handled separately. Representative specimens were taken for histological examination routinely. Except the proliferation of the dilated cysts in the liver of rat fed on AAF, the changes in the liver are similar to those seen in the livers of rats fed on DAB as described by the previous investigators in the other reports of this series (2, 3).

The samples of the liver tissue taken for the examination of the guanase and arginase were cut into several pieces.

a) Estimation of guanase.

Substrate: 100 mg of guanine hydrochloride (Zellstoffabrik Waldhof, Germany) was dissolved in 20 ml of distilled water with a few drops of 33 per cent NaOH solution. Then 50 ml of 0.5 per cent gelatine solution was added. After neutraliz-

ed with 33 per cent of HCl, gelatine solution was added to this solution to make the whole volume up to 100 ml.

Enzyme extract: The hepatic tissues were excised from the animal immediately after decapitation and exsanguination, weighed and homogenized. Then the distilled water was added to the tissue paste exactly to 25 times its original tissue volume (ml) calculated from the weight (mg) and was thoroughly shaken and centrifuged.

Estimation of guanase activity: 5 ml of the substrate, 1 ml of the enzyme extract, 10 ml of buffer solution—citrate buffer (pH 5), phosphate buffer (pH range 6 to 8) and glycine-NaOH buffer (pH range 9 to 11)—and 4 ml of the distilled water were transfered into a large test tubes (3.4×21.3) which were stoppered tightly and were stored at 37°C for 3 hours. The ammonia liberated during the incubation was absorbed into 20 ml of N/50 H_2SO_4 solution through Folin's apparatus. Adding 5 ml of Nessler's reagent to 5 ml of the treated sulfuric acid and the color of the solution was estimated photometrically. The blank test was carried out at the same time.

b) Estimation of arginase

Substrate: The M/10 solution of arginine hydrochloride (Ajinomoto) in glycine-NaOH buffer (pH 9.5) was used.

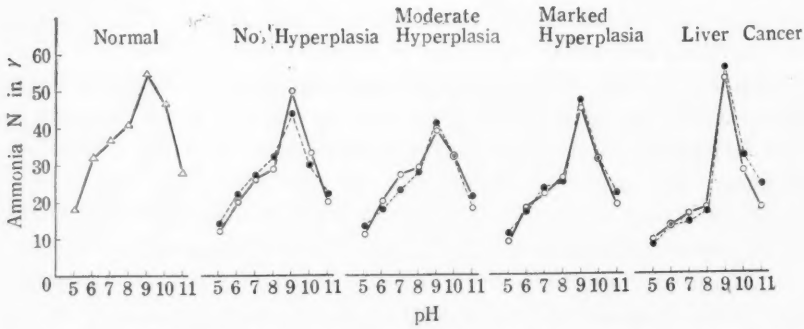

Fig. 1 Graphs showing the guanase activities of the liver of rats fed AAF or DAB (protted from Table 1).
Triangle: Normal, Circle: AAF, Solid circle: DAB.

Enzyme extract: 50 times of the distilled water was added to the liver tissue, homogenized and then centrifuged.

Estimation of arginase activity: 5 ml of substrate, 1 ml of the enzyme extract 3 ml of buffer solution and 3 ml of distilled water was transferred into a large test tube (3.4×21.3). After adding a small amount of toluene, each test tube was stoppered tightly and incubated at 37°C for one hour. After the incubation, the liberated urea was determined by urease method routinely.

Results

1) Experiment on guanase

The two carcinogens, AAF and DAB, induced almost a similar change in the content of the guanase in the hepatic tissues in relation to the histologic changes. The mean values and the ranges of the guanase of the liver of the rats fed on AAF, on DAB and normal rats corresponding to the various acidities are shown in Table 1.

Table 1. Guanase Activity of the Liver of Rats Fed AAF or DAB at Various Acidities
Numbers, mean value with maximum and minimum values, represent ammonia nitrogen in γ

Liver Findings		No. of Experiments	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11
Normal		9	18 (15–26)	32 (24–42)	37 (27–46)	41 (32–48)	55 (39–67)	47 (37–58)	28 (16–43)
AAF	No Hyperplasia	4	12 (8–18)	20 (15–27)	26 (21–31)	29 (22–34)	50 (44–57)	33 (25–50)	20 (14–25)
	Moderate Hyperplasia	12	11 (7–15)	20 (16–27)	27 (12–34)	28 (20–35)	39 (24–48)	32 (20–39)	21 (16–26)
	Marked Hyperplasia	11	9 (5–15)	18 (13–28)	22 (16–32)	26 (19–39)	45 (29–61)	31 (21–49)	19 (9–28)
	Liver Cancer	7	9 (7–16)	13 (10–20)	15 (11–28)	17 (11–24)	53 (41–63)	28 (21–34)	18 (13–24)
DAB	No Hyperplasia	11	14 (9–20)	22 (15–28)	27 (20–34)	32 (22–44)	44 (35–57)	30 (22–39)	22 (12–30)
	Moderate Hyperplasia	9	13 (7–20)	18 (10–24)	23 (14–28)	28 (19–32)	41 (27–55)	30 (18–41)	18 (10–28)
	Marked Hyperplasia	10	11 (8–18)	17 (11–25)	23 (15–29)	24 (17–34)	47 (30–59)	31 (19–48)	22 (10–32)
	Liver Cancer	8	8 (5–12)	13 (9–18)	14 (10–21)	16 (13–24)	56 (35–68)	32 (25–41)	24 (19–38)

As is shown in the table, generally speaking, there was no significant difference in the value of the guanase between the two groups of carcinogen-fed rats and control rats at pH 9, which is known as the optimum acidity for this enzymes. On the other hand, at each range of the other acidities examined (pH 5, 6, 7, 8, 10 and 11) except pH 9, a considerable decrease is found in the guanase activity of the liver when the tissues are non-cancerous. While the activity of the guanase was dropped to only one-half of the control when the tissue have become cancerous.

2) Experiment on arginase

The mean values and the ranges of the arginase activities of the hepatic

Table 2. Arginase Activity of Liver of Rats Fed AAF
Figures, mean value with maximum and minimum values, reporesent N/100 NaOH consumed in ml.

Liver Findings	No. of Experiments	Activity of Arginase	※ Relative Activity	Activity of Arginase in the Presence of Manganium sulfate (M/200)
Normal	6	24.8 (22.7–29.0)	100	36.7 (34.5–40.1)
No Hyperplasia	9	22.6 (19.1–25.4)	91	27.1 (23.1–35.6)
Moderate Hyperplasia	21	25.8 (22.1–34.5)	104	30.9 (25.1–38.2)
Marked Hyperplasia	24	22.8 (19.0–29.9)	91	27.3 (21.7–36.8)
Liver Cancer	17	4.4 (1.0–7.8)	17	6.9 (1.5–11.7)

※ Value for normal taken as 100.

tissues at pH 9.5 are shown in Table 2. In the table, it is clearly seen that the relative degree of arginase activity of the livers which showed no hyperplasia

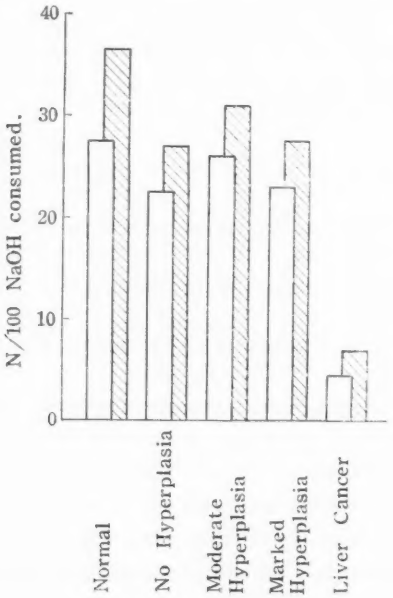

Fig. 2 Arginase activity of liver of rats fed AAF. Shaded area represents the Arginase of the liver in the presence of Manganium sulfate.

and moderate hyperplasia were 91 and 104 respectively, whereas the activity of the normal liver reached just 100. The arginase activity of the marked hyperplasia was 91, and that of liver cancer was only 17. This indicated that the

arginase activity of rat liver was slightly decreased once and then increased when the pathological change in the liver was non-cancerous. But, when the liver tissue became cancerous, the arginase activity dropped to less than one-fifth of normal.

In the presence of manganium sulfate (M/200) the rate of arginase is increased in all examples.

Discussion and Conclusion

It is known that guanine and arginine have a close association with the metabolism of the nucleus and it has become of interest to determine the guanase and the arginase values preceding or following the development of tumors in the liver during development of a neoplasm.

It has been shown that tumors of the liver in the rat have a lower arginase concentration than normal liver (4–6, 11–13). And Greenstein, working with transplanted hepatoma (7–9, 11–13) found that guanase in the tumor tissue was almost the same concentration to that of normal rat liver.

In the present study, the guanase activity of the liver tissue at optimum acidity (pH 9) showed no relation to the progressive pathologic changes in the liver brought about by feeding carcinogens to rats. But at pH 5, 6, 7, 8, 10 and 11 the content of the guanase in the liver decreased gradually in the process of the hepatic changes and dropped to only one-half of the control when the tissue has become cancerous.

On the other hand, the amount of arginase present in hepatic tissue decreased definitely in the course of pathological change from normal to neoplastic hepatic tissue.

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要　旨

肝癌生成過程における白鼠肝グァナーゼおよびアルギナーゼについて

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2-Acetylaminofluorene (AAF) あるいは p-Dimethylaminoazobenzene (DAB) を白鼠に与え，実験的に肝癌を生成する過程における肝のグァナーゼおよびアルギナーゼの活性度を測定検討した。

最適酸度 (pH 9) におけるグァナーゼ活性度は，正常肝より肝癌に至る病変の進行過程には無関係にほとんど不変であったが，他の酸度における活性度は病変の進行に応じてかなり減じてゆき，肝癌では正常肝の $1/2$ 程度の活性度を示すにすぎなかった。

アルギナーゼ活性度については，従来の多くの報告と同じく病変の進行に応じて漸減し，肝癌では正常肝の $1/5$ を示した。しかして硫酸マンガンの存在の下では顕著に賦活された。

(厚生科学研究費による)

[GANN, Vol. 45; December, 1954]

STUDIES ON RHODANESE OF THE LIVER OF RATS DURING HEPATIC CARCINOGENESIS

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Many attempts to study the activities of the several enzymes of the liver of rats in the course of the hepatic carcinogenesis have been made in the laboratories of the Biochemistry and Medical Zoology of this School (1-5). We have now conducted a study of the relative activities of rhodanese during the course of the production of liver cancer (6, 7), and the details of this experiment are given in the following lines.

EXPERIMENTS

One hundred and twenty male Wistar rats about 2 months old, 60-80 g in weight, were used for the experiment. Sufficient carcinogen was incorporated in the polyethylene glycol fraction to bring the final concentration of the carcinogen in the diet to 0.05 per cent for 2-acetylaminofluorene (AAF), or 0.06 per cent for p-dimethylaminoazobenzene (DAB). One group of 60 animals was kept on the diet containing AAF for 5 months, and a second group of 60 animals on DAB for 4 months so as to produce liver cancer. Rats in both groups were returned to a normal rice diet without any carcinogen for additional months.

Rats in the first group were killed at 7 months and the second group at 5 months after the experiment was began. All livers were carefully examined for tumors at the time of autopsy. Each sample of liver taken for enzymatic determination was placed in one of the following categories, depending upon the type of hepatic tissue changes: 1) no hyperplasia, 2) slight hyperplasia, 3) moderate hyperplasia, 4) marked hyperplasia and liver cancer. These designations closely correspond to those described by Wilson and associates (8), and the identification of these liver findings has been described in publication cited (1, 2). If the liver contained both cancerous areas and relatively normal tissues, the different portions were dissected out and handled separately.

The hepatic tissues were excised from the animal after decapitation and exsanguination, weighed and homogenized. Then the distilled water was added to the tissue paste exactly to 100 times its original tissue volume (ml) calculated from the weight (g) and was centrifuged. The aliquot volume of the liver extract was used for enzyme assay.

The reaction mixture consisted of 1 ml of 0.1 M KCN, 1 ml of 0.2 M $Na_2S_2O_3$,

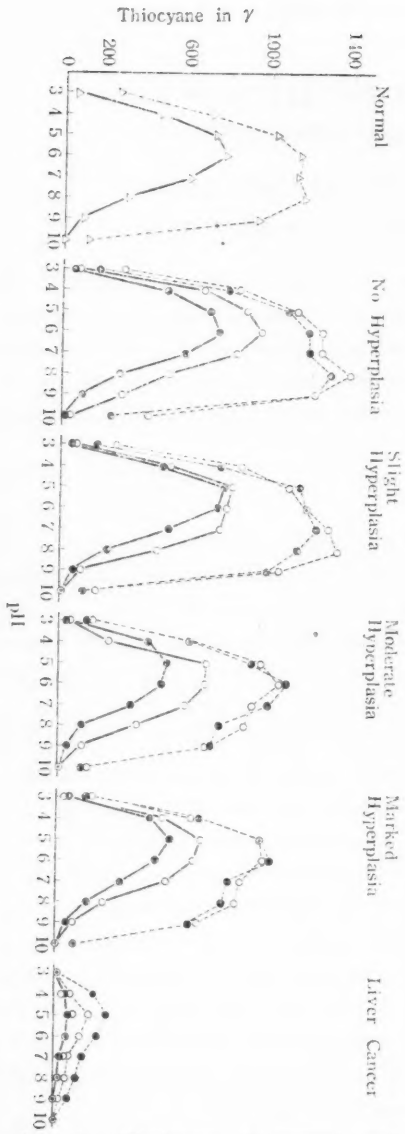

Fig. 1. Rhodanese of normal and pathological rat liver. Tissue rhodanese plotted against the pH at which the determinations were made.

Triangle: Normal liver. Circle: Livers of rats fed AAF. Solid circle: Livers of rats fed DAB. The broken line represents the rhodanese of the liver in the presence of cysteine.

3 ml of buffer solution (0.1 M veronal-acetate buffer pH range 3 to 9, 0.1 M glycine-NaOH buffer pH 10 to 11), 1 ml of 100 times diluted liver suspension and 4 ml of distilled water. The reaction was carried out at 37°C, for 1 hour, after which 5 ml of the reagent (50 g of conc. HNO_3 and 25 g $Fe(NO_3)_3$ in 500 ml of distilled water) was added to 5 ml of the mixture for the estimation of the thiocyanate. This colored solution was filtered to remove protein precipitated and subjected to photocolorimetry.

The mean values and ranges of the activities of rhodanese of the liver of carcinogen-fed and normal animals at corresponding acidities are shown in Table 1 and Figure 1. In the liver of a normal rat, there is a considerable rhodanese with the maximum of activity around pH 6. And in the presence of cysteine the activity of rhodanese is increased markedly with a broad maximum between pH 6 and 9. It may be seen that the rhodanese of the liver of AAF-fed rats showed always a higher value in its activity than that of DAB-fed rats during the course of the production of liver cancer. The activities of the rhodanese of the livers of carcinogen-fed rats decreased gradually in the process of liver cancer production. It is noteworthy that the activity of the rhodanese of liver cancer is greatly decreased with or without the addition of cysteine to the reaction mixture. The mean value of the activity of liver cancer was one-eighths at pH optimum in AAF-fed rats and one-twelfths in DAB-fed rats. A considerable change in the acidity of maxima of enzymatic

Table 1. Rhodanese activity of the liver of rats fed AAF or DAB at various acidities.
Numbers, mean value with maximum and minimum values, represent thiocyane formed in γ.

	Liver Findings	No. of Experiments	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
	Normal	10	61 (35–110)	475 (377–512)	732 (630–850)	776 (548–992)	615 (332–730)	318 (160–425)	104 (43–145)	5 (0–10)
			*265 (90–398)	712 (670–760)	1026 (970–1065)	1151 (1100–1185)	1132 (1000–1200)	1176 (1065–1310)	958 (792–1370)	124 (70–225)
AAF	No Hyperplasia	5	79 (35–110)	682 (483–806)	886 (806–1000)	961 (850–1030)	845 (555–970)	511 (259–720)	281 (212–383)	26 (10–39)
			*293 (100–430)	845 (820–865)	1135 (1065–1170)	1254 (1235–1270)	1260 (1200–1310)	1390 (1370–1430)	1235 (1110–1420)	412 (325–555)
	Slight Hyperplasia	5	72 (39–120)	519 (455–588)	809 (730–880)	803 (670–1000)	796 (700–940)	471 (325–730)	86 (48–140)	14 (0–35)
			*267 (90–433)	871 (770–1170)	1109 (1030–1170)	1193 (1130–1235)	1285 (1235–1370)	1341 (1235–1410)	1052 (940–1170)	167 (70–237)
	Moderate Hyperplasia	10	48 (32–100)	237 (455–630)	719 (610–940)	717 (570–940)	609 (340–910)	384 (150–710)	118 (52–248)	15 (0–35)
			*151 (90–225)	733 (670–865)	981 (895–1130)	1071 (985–1170)	943 (880–1235)	905 (730–1210)	738 (383–940)	133 (30–210)
	Marked Hyperplasia	10	36 (0–90)	513 (305–630)	697 (383–940)	667 (370–895)	541 (212–750)	216 (52–370)	70 (0–105)	5 (0–20)
			*165 (140–190)	650 (630–670)	978 (940–1015)	993 (970–1025)	893 (670–1115)	885 (740–1030)	682 (483–880)	95 (20–170)
	Liver Cancer	5	4 (0–10)	37 (0–80)	94 (0–115)	63 (0–90)	51 (0–100)	20 (0–43)	8 (0–20)	3 (0–10)
			* 21 (0–43)	89 (0–237)	172 (0–323)	126 (0–259)	62 (0–120)	47 (0–80)	22 (0–35)	3 (0–10)

DAB	No Hyperplasia	6	53 (43–70)	496 (455–540)	703 (588–750)	736 (640–792)	586 (383–700)	271 (140–410)	90 (52–170)	9 (0–20)
			*165 (130–200)	800 (781–820)	1089 (1048–1130)	1194 (1170–1218)	1193 (1185–1200)	1297 (1253–1340)	1227 (1200–1235)	230 (212–248)
	Slight Hyperplasia	4	48 (35–52)	496 (470–527)	789 (670–985)	771 (610–985)	509 (318–670)	220 (150–259)	63 (43–90)	4 (0–10)
			*175 (170–180)	770 (750–790)	1159 (1100–1218)	1183 (1130–1235)	1243 (1115–1370)	1117 (1000–1235)	1000 (690–1310)	103 (65–140)
	Moderate Hyperplasia	4	35 (28–43)	436 (298–512)	505 (225–650)	503 (270–720)	353 (180–455)	107 (52–150)	41 (20–52)	12 (5–10)
			*130 (110–159)	725 (680–742)	940 (910–970)	1100 (980–1210)	1015 (980–1095)	770 (580–895)	750 (398–910)	110 (55–170)
	Marked Hyperplasia	12	42 (20–60)	448 (248–555)	553 (180–720)	486 (120–670)	328 (65–483)	145 (52–212)	48 (10–110)	2 (0–20)
			*154 (125–195)	688 (640–750)	970 (940–1000)	1030 (1000–1120)	827 (710–1015)	800 (539–985)	651 (212–925)	86 (20–135)
	Liver Cancer	8	0	57 (0–70)	64 (0–90)	52 (0–70)	36 (0–60)	7 (0–28)	0	0
			* 15 (10–20)	199 (160–237)	262 (248–483)	214 (180–298)	150 (120–180)	130 (75–185)	66 (43–90)	5 (0–10)

* Activity of rhodanese in the presence of cysteine hydrochloride (M/1000).

activities suggests the enzyme may be altered in amount or in kind.

Summary and Conclusion

The rhodanese amount of the hepatic tissue and hepatoma was determined in rats fed hepatic carcinogens, 2-acetylaminofluorene or p-dimethylaminoazobenzene. The two carcinogens, AAF and DAB, induced almost a similar change in the content of the rhodanese in the hepatic tissues in relation to the histologic changes. The amount ot the enzyme contained in the hyperplastic livers was always lower than that or control livers corresponding to the various acidities, while the rhodanese activity in the case of liver cancer dropped definitely. There was a considerable change in the acidity of maxima of enzymatic activities during the course of the production of liver cancer. It suggests that the enzyme may be altered in amount or in kind in the pathological tissue as compared to normal.

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要　旨

肝癌生成過程における白鼠肝ロダネーゼについて

一井昭五，稲葉正子，森　和雄
(昭和医科大学　医動物学教室)

2-Acetylaminoflourene (AAF) あるいは p-Dimethylaminoazobenzene (DAB) を白鼠に与え実験的肝癌生成過程における肝ロダネーゼの活性度を測定した。

初期の病変を示す肝ロダネーゼ活性度は正常肝に比し大差が認められなかったが，硬変を呈した肝ロダネーゼはかなり低い活性度を示した。　さらに癌化した肝のロダネーゼは正常肝の $1/3$〜$1/12$ 程度を示すにすぎなかった。

活性度の最高を示す酸度が肝の病変に応じて酸性域に移動した事実から肝癌生成過程における白鼠肝ロダネーゼは量的並びに質的に変化することを暗示している。

(厚生科学研究費による)

[GANN, Vol. 45; December, 1954]

EFFECT OF K. I. K. FACTOR ON VARIOUS LIVER ENZYMES OF ANIMALS (1st Report)

RYUZO IWATSURU, ISAO KATO and HARUHIKO TAMAKI
(Wakayama Medical College)

I. On Catalase

It has long been presumed that the cachectic state inevitable in cancer bearing animals is attributable to some toxic substance from cancerous tissue, and so a lot of studies have been accomplished about this toxic substance or so-called "cancer toxin".

However, on surveying the reports, one finds considerable varieties of toxins according to each investigator, their properties differ as well as their effects, which fact leads to the assumption that these "toxins" are not unitary but various different toxins can be obtained from cancer tissue.

In 1937, Iwatsuru also discovered an anemiogenic substance in the gastric juice of gastric cancer patients, and named this as K. I. K. factor, and this K. I. K. factor is non-dialysable, thermostable, water soluble but not soluble in ether or in alcohol, and not precipitable by trichloracetic acid.

Meanwhile, the extensive works about the effect of tumor growth on tissue enzymes of animals were reported by Greenstein (1947), and recently successful studies in this field were performed by many investigators.

On catalase, Blumenthal (1910) and Rosenthal (1912) first reported that the liver catalase activity was markedly reduced in cancer-bearing animals, and this was ratified by many investigators. In human beings also Brahn (1916) demonstrated the decreased liver catalase activity in the cancer corpse. Zerner (1923), and Deutsch and Fraenkel (1933) reported the low blood catalase value of the patients with cancer, while Yanagizawa (1942), Nakahara and Fukuoka (1944) and Mori (1952) confirmed the depressed liver catalase in the course of experimental liver cancer production.

Nakahara and Fukuoka (1948), furthermore, discovered that alcohol precipitate of the extract from cancer tissue, namely, their toxohormone, displayed marked depression of liver catalase in mice. This toxohormone is, they reported, non-dialysable, water soluble, but not soluble in ether or alcohol, and precipitable by trichloracetic acid, picric acid, etc. Recently they reported two splendid contributions, that is, 1. Success in biosynthesis of toxohormone in vitro from several amino acids, and 2. Possibility of the existence of dialysable form of toxohormone.

Nakagawa and his co-workers for many years have reported numerous contributions about cancer toxin found in the urine of patients with cancer; their "catalase factor" has also depressing effect on liver catalase of mice.

Sato and Yuki (1951) produced a certain polypeptide fraction from cancer extract, which proved both anemiogenic and catalase-depressing effects on mice.

Considered from the above mentioned reports, K. I. K. factor, toxohormone, Nakagawa's catalase factor and Sato's polypeptide fraction are all originated from cancer tissue and have similar properties. Thereupon it has become important for us to find out whether K. I. K. factor depresses the catalase or not.

Experiment 1. The material we used was prepared in the same way we reported in the former report, and 1 cc of the material is equivalent to 30 cc of the cancerous gastric juice. To the rat weighing ca. 100 g, 1 cc of the material was injected. After 24 hours the rat was killed by decapitation.

Estimation of catalase activity was performed with the method used by Nakahara and Fukuoka, but the liver catalase of rat was scanty as compared with that of mice, so we used 1 cc of 100 times diluted enzyme solution.

Results

1. Liver catalase activity of 30 normal rats: max. 29.3, min. 14.4, average 21.06

2. In 23 rats injected with methanol precipitate of cancerous gastric juice: max. 15.1, min. 6.0, average 11.01.

3. In 13 rats injected with methanol precipitate of non-cancerous gastric juice: max. 29.2, min. 16.2, average 20.01.

These findings are summarized in Fig. 1.

Experiment 2. To the concentrated cancerous gastric juice the same volume of 10% trichloracetic acid was added and after centrifugation the precipitate was dissolved in a small quantity of N/10 NaOH. Both dissolved precipitate and supernatant were dialysed for 2 days, and then the supernatant was again concentrated to 2-3 cc. Each of these materials was injected to rats, and similarly as in Experiment 1, liver catalase was estimated.

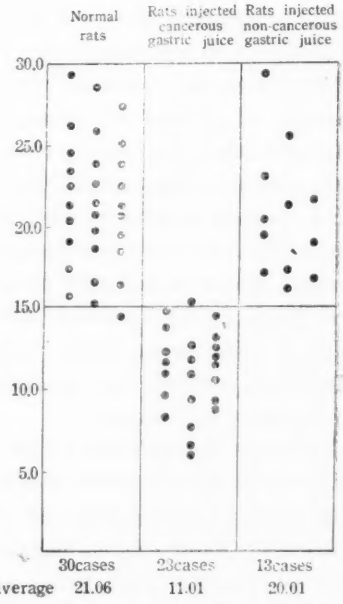

Fig. 1. Effect of Methanol Precipirate from Cancerous and Non-cancerous Gastric Juice on Liver Catalase of Normal Rats.

Results

As shown in Table 1, trichloracetic acid precipitate of cancerous gastric juice depressed catalase activity, but supernatant had no such effect. As control, four non-cancer materials were tested, but both precipitate and supernatant proved no depressing effect.

Table 1. Effect of Trichloroacetic Acid Supernatant and Precipitate of Cancerous Gastric Juice on Liver Catalase Activity of Normal Rats.

No	Catalase activity	
	Supernatant	Precipitate
1 (Cancer)	17.5	12.9
2 (〃)	18.2	13.4
3 (〃)	16.0	11.6
4 (〃)	20.9	9.2
5 (〃)	23.6	8.3
6 (Normal)	18.8	21.1
7 (〃)	17.9	18.2
8 (〃)	23.0	16.5
9 (〃)	16.1	20.4

Summary and Conclusion

Methanol precipitate of gastric juice from gastric cancer patients presents evidently depressing effect on liver catalase of rats. In this methanol precipitate, both K. I. K. factor and toxohormone are contained, so the depressing effect on catalase of this methanol precipitate does not necessarily mean that K. I. K. factor displays that effect. The lowered catalase activity may be attributed to the co-existing toxohormone.

In Experiment 2, we separated K. I. K. factor from toxohormone by aid of trichloracetic acid, for this reagent precipitates toxohormone as applied for the purification of this substance by Nakahara and Fukuoka, but K. I. K. factor is not precipitated by it as Yutani had previously reported.

We examined the catalase depressing effect of the trichloracetic acid supernatant (K. I. K. factor fraction) and of the precipitate (toxohormone fraction) and the result was that the K. I. K. factor fraction had no catalase depressing effect, while the toxohormone fraction naturally proved to depress the catalase. Thus we ascertained that K. I. K. factor and toxohormone are decidedly different substance, though they are otherwise alike in many of their properties.

II. On Phosphatase

About the phosphatase of tumor bearing animals, Woodard (1943) reported that the liver of the rats with cancer showed higher phosphatase activity than that of normal rats. Ichii, Mori and Ohashi (1954), in experimentally produced liver cancer, also found high value of alkaline phosphatase. Eguchi (1951) too found in the urine of patient with cancer a substance which by injection to mice increase the level of alkaline phosphatase of liver tissue.

We also investigated the effect of cancerous gastric juice on the acid and alkaline phosphatase of rats liver, and attained the unforseen result which is contary to the above mentioned contributions.

Experiment 1. The material, the mode of injection, and the rats used for the experiment are similar to those of the previous experiments (on catalase). 24 hours after the injection rat is killed by bleeding, and immediately after the death liver is excised, and 1 g of it is homogenized. To this, 100 cc of aq. dist. is added, and after stirring, centrifugated, and 1 cc of supernatant is employed as enzyme solution. We used Key's method modified by Shirai for the estimation of phosphatase.

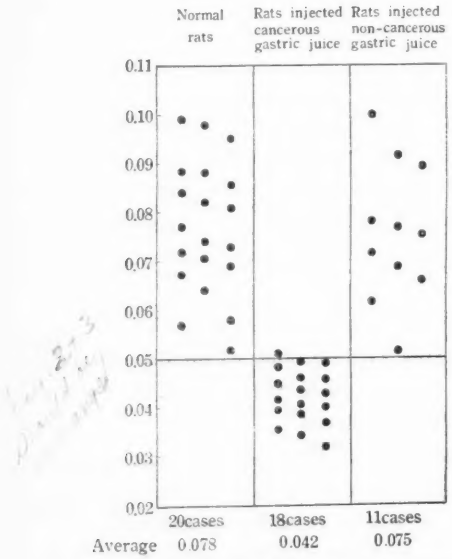

Fig. 2. Effect of Methanol Precipitate of Cancerous and Non-cancerous Gastric Juice on Acid Phosphatase of Normal Rat Liver.

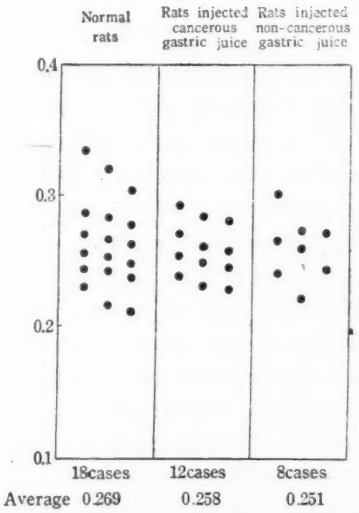

Fig. 3. Effect of Methanol Precipitate of Cancerous and Non-Cancerous Gastric Juice on Alkaline Phosphatase of Normal Rat Liver.

Results

The results are shown in Fig. 2 (acid pho.) and in Fig. 3 (alkaline pho.). Acid pho. activity of liver does not show any change despite the methanol precipitate injection of cancerous gastric juice, but alkaline pho. is markedly lowered by injection of methanol precipitate of cancerous gastric juice.

Experiment 2. As in the case of catalase, trichloracetic acid precipitate and supernatant are used as material, and the methods are the same as in Experiment 1. The results are summarized in Table 2; namely both supernatant and

Table 2. Effect of Trichloroacetic Acid Supernatant and Precipitate of Cancerous Gastric Juice on Alkaline phosphatase of Normal Rat Liver.

No	Phosphatase activity	
	Supernatant	Precipitate
1 (Cancer)	0.068	0.048
2 (〃)	0.081	0.032
3 (〃)	0.071	0.047
4 (〃)	0.053	0.040
5 (〃)	0.089	0.035
6 (Normal)	0.076	0.084
7 (〃)	0.092	0.054
8 (〃)	0.098	0.060

precipitate by trichloracetic acid of cancerous gastric juice show no effect on acid pho., and on alkaline pho. supernatant (K. I. K. factor fraction) also show no effect, but precipitate decidedly lowers the activity.

Summary and Conclusion

We found that the methanol precipitate of cancerous gastric juice reduces the alkaline phosphatase of rat liver, and also the trichloracetic acid precipitate of it has the same effect, but trichloracetic acid supernatant which corresponds to the K. I. K. factor shows no effect on liver phosphatase. We showed that cancerous gastric juice indeed has catalase depressing effect and also has alkaline phosphatase depressing effect, but these effects are not caused by K. I. K. factor.

There may be many different "cancer toxins" in the cancerous gastric juice, and though chemical and physical properties of them may roughly be in accord, their biological effects markedly differ, and more evidence will be produced by the successful purification of each toxin.

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要　旨

K. I. K. 因子の肝諸酵素に及ぼす影響について（第一報）

岩鶴龍三，加藤　績，玉置治彦

（和歌山医大　第一内科）

1. 胃癌胃液のメタノールによる沈澱（トキソホルモン及び K. I. K. 因子を含む）及び三塩素醋酸による沈澱（トキソホルモンに相当）は，これを注射することにより，ダイコクネズミの肝カタラーゼ値を低下せしめるが，三塩素醋酸で沈澱しない部分（K. I. K. 因子に相当）はこの作用を有しない。

2. 胃癌胃液のメタノールによる沈澱及び三塩素醋酸による沈澱は，これを注射することによりダイコクネズミの肝アルカリ性フォスファターゼ力価を低下せしめるが，三塩素醋酸で沈澱しない部分（K. I. K. 因子に相当）はこの作用を有しない。

（文部省科学研究費による）

Book Review

ATLAS OF EXFOLIATIVE CYTOLOGY

By George N. Papanicolaou. 106 pp, 36 color plates. Published for the Commonwealth Fund, Harvard University Press, Cambridge, Mass. 1954.

The founder of and top-authority on the exfoliative cytology has given to the students of this practical field a color atlas with 36 plates of most excellent technical reproduction. Although the author modestly admits that our present knowledge of this newly explored cytologic field is still limited and an atlas can be but incomplete, and that he prefered to give in a loose-leaf style to facilitate adding further notes, this is a complete volume beginning with techniques for collecting materials and ending in highly specialized differential diagnostic discussion, covering all fields accessible to the exfoliative cytology, female and male genital organs, urinary system, respiratory and digestive tracts, breast, and serous exduates.

Detailed description and abundant illustrations will help even an expert to further knowledge of exfoliated cells, both physiologic and pathologic. Much space has been dedicated to present variegated types of normal components and their wide ranges of variability in each system concerned, acquaintance of which is a prerequisite to an adequate diagnostic evaluation.

Most of the illustrations are by the hands of an expert artist of this field, Mr. Hashime Murayama, who is our compatriot and has been associated with the author for many years. A number of drawings, which appeared in the earlier publications by the same author, were also included. This makes the present volume a complete handbook, an atlas and a reference book at the same time, covering the entire field of exfoliative cytology. Bibliography is comparatively limited to a number of 48 selected representative works.

K. Oota, Cancer Institute, Tokyo.

日本癌学会・癌研究所推薦

癌の知識

東京医科歯科大学教授
癌研病理部長・医博　太田邦夫著

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癌を一般に正しく認識してもらうことこそ現代癌対策の第一目標である。本書はその目的のために，癌とはどんなものか・癌をもった人ともたない人・動物の癌・癌はどうしてできる・癌は遺伝するか・癌とビールス・癌の発生と治療・社会的癌対策等についてできるだけ分け易く記した権威ある解説書である

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酵素化学の進歩　5集の1

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酵素の必須原子団（丹羽允）・Coenzyme A の生化学（赤沢堯）・銅を含む酸化酵素（本田幸一郎・三浦一夫）・物質代謝から見た抗生物質の作用機作（笹川泰治）の諸編を収めたもので，本集より研究成果の早期発表のために分冊として読者の便をはかった学生，研究者に絶好の最新参考文献である。

放射性追跡子実験技術

シュビツア・ホイットニー　著
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放射性物質取扱い者の必読書として丁寧かつ具体的に説いたもので，放射性物質の取扱い注意・研究室の構造・研究者の動作から基礎・化学・物理学・生物学的の各種実験例を掲げて詳細に記されている。この方面に実際に従事している人は勿論，この方面に関心を有する人の一読すべき好参考書である。

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癌　第45巻　第4号

昭和29年12月25日　印刷
昭和29年12月31日　発行

編集兼発行代表者　中原和郎　東京都豊島区西巣鴨2丁目

印刷者　加藤保幸　東京都千代田区神田三崎町2ノ12

印刷所　株式会社 加藤文明社　東京都千代田区神田三崎町2ノ12

発行所　財團法人癌研究会・日本癌学会　東京都豊島区西巣鴨2丁目　電話 池袋(97) 5186番 5187番

取扱店　東京都千代田区神田駿河台3ノ9　電話 神田 (29) 2951～3 番　共立出版株式会社